Optimising the Use of Misoprostol for the Prevention of Postpartum Haemorrhage

Thesis submitted in accordance with the requirements of the University of Liverpool for the Degree of Doctor in Philosophy by

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Declaration

I declare that the studies and reviews presented in this thesis are the results of my own independent work, unless otherwise acknowledged.

The content of this thesis has not and is not being currently submitted for candidature for any other degree.

Anisa Elati

Dedication

This thesis is dedicated to ***Prophet Muhammad*** peace be upon him.

He is the man who taught us patience in the face of adversity, and taught us to live happily in this world but seeking the eternal life in the hereafter.

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Published papers and abstracts

Published Papers/ Articles

1. **Elati, A.** and Weeks, A. (2009), **The use of misoprostol in obstetrics and gynaecology**. BJOG, 116: 61–69. doi: 10.1111/j.1471-0528.2009.02329.x

2. **Elati A**, Elmahaishi MS, Elmahaishi MO, Elsraiti OA, Weeks AD (2011) **The effect of misoprostol on postpartum contractions: a randomised comparison of three sublingual doses.** BJOG, 118,4, 466-473
3. **Elati, A** and Weeks, A (2011), **Misoprostol for the management of postpartum haemorrhage**. BMJ, 342:d2877

4. Patel, D., Nasir, S., **Elati, A.,** Vernon, G. and Weeks, A. (2011), **Historical trends in the timing of informed consent for research into intrapartum complications.** BJOG, doi: 10.1111/j.1471-0528.2011.03204.x

5. **Elati A** and Weeks A. **The risk of fever after administration of misoprostol for prevention of postpartum haemorrhage: systematic review and meta-analysis** (accepted in Obstetrics and Gynaecology)

Presentations at international/ national meetings

1. **Elati A**, Weeks A. **A randomised comparison of three misoprostol doses for postpartum myometrial contraction.** The North of England Obstetrical and gynaecological Society meeting, Liverpool, UK, March, 19, 2010. **(Oral presentation)**
2. **Elati A**, Weeks A. **The effect of misoprostol on postpartum contractions: a randomised comparison of three sublingual doses.** British Maternal and Fetal Medicine Society, 14th Annual Conference, Newcastle, UK, June, 11-12, 2010. ***(Oral presentation)***
3. **Elati A**, Weeks A. **A randomised comparison of three doses of sublingual misoprostol on postpartum myometrial contractility**. 32nd British International Congress of Obstetrics and Gynaecology, Belfast, UK, June, 20 – 23, 2010. ***(Oral presentation)***
4. **Elati A**, Weeks A. **The effect of misoprostol on postpartum contractions: a randomised comparison of three sublingual doses.** 15th annual international Conference of the Obstetrics & Gynaecology Department – Ain Shams University, Cairo, Egypt, May, 25-26, 2010. ***(Oral presentation)***
5. **Elati A. Obstetrics Haemorrhage.** Maternal Mortality Teaching Day Birmingham, May, 18, 2010. ***(Oral presentation)***
6. **Elati A and** Weeks A. **The risk of fever after administration of misoprostol for prevention and treatment of postpartum haemorrhage: systematic review and meta-analysis.** The North of England Obstetrical and gynaecological Society meeting, Liverpool, UK, March, 18, 2011. ***(Oral presentation)***
7. **Elati A and** Weeks A. **The risk of fever after administration of misoprostol for prevention and treatment of postpartum haemorrhage: systematic review and meta-analysis.** Liverpool, UK, PRISM 2011. ***(Poster presentation)***
8. **A Elati**, W Leon , J Durocher , B Winikoff, A Weeks, and A Alfirevic. **An association study of genetic polymorphisms and misoprostol induced fever** The Annual Academic Meeting at Royal College of Obstetricians and Gynaecologists. London, December, 7, 2011. ***(Oral presentation)***

List of abbreviations

ADRB1,2,3	Adrenergic, beta, receptor
ADRBK1	Adrenergic, beta, receptor kinase 1
AMTSL	Active management of the third stage of labour
ANOVA	Analysis of variance
AP	The area postrema
APH	Anti-Partum Haemorrhage
API	Pressure integral
ATPase	Adenosine triphosphatase
AU	Alexandria Units
AUC	Area Under the curve
BAT	Brown adipose tissue
cAMP	Cyclic adenosine monophosphate
CCT	Controlled cord traction
Cmax	The peak concentration
COX	Cycloxygenase
CVOs	Circumventricular organs
D&C	Dilatation and curettage
dbSNP	SNPs database
DHP	Dihydropyridine calcium channel
DHP-R	Dihydropyridine receptor
DIC	Disseminated intravascular coagulation
DMH	Dorsomedial hypothalamus;
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immuno Sorbent Assay
EP1, EP2, EP3, EP4	Prostaglandin receptors
FDA	Food and Drug Administration
FIGO	International Federation of Gynaecology and Obstetrics

GABA receptors	Gamma-aminobutyric acid receptor
GWA	Genome-wide association
GWAS	Genome wide association studies
Hb	Haemoglobin
ICM	International Confederation of Midwives
ICV	Intracerebroventricular
IL1-β	Interleukin 1 beta
IL-6	Interleukin 6
InsP3	Inositol 1, 4, 5-trisphosphate
IOL	Induction of labour
IUFD	Intrauterine fetal death
IUP	Intrauterine pressure
LD	Linkage disequilibrium
LOA	limits of agreements
LPS	Lipopolysacharides
MAF	Minor allele frequency
MALDI-TOF MS	Matrix Assisted Laser Desorption/Ionisation-Time-of-Flight Mass Spectrometry
MAV	Mean active pressure
MH	Malignant hyperthermia
MMR	Maternal mortality rate
MnPO	Median preoptic area
MOAT	Multi-specific organic anion transporter
MPA	Misoprostol acid
MTT	4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide, a yellow tetrazole assay)
MVU	Montevideo unit
MVA	Manual vacuum aspiration

NCBI	The National Center for Biotechnology Information
NICE	The National Institute for Health and Clinical Excellence
NSAIDs	Non-Steroidal Anti inflammatory Drugs
OATP2A1, OATP1B1	Organic anion transporter family, member
OR	Odd ratio
OVLT	Organum vasculosum of the laminae terminalis
PBMCs	Peripheral blood mononuclear cells
PGF2α	Carboprost
PGs	Prostaglandins
PGT	Prostaglandin transporter
PLC	Phospholipase C
POAH	Anterior hypothalamic preoptic area
PPH	Postpartum haemorrhage
PROM	Premature rupture of membrane
PT	Prothrombin time
PTT	Partial thromboplastin time
r^2	Correlation coefficient
RCOG	Royal College of Obstetricians and Gynaecologists
RCT	Randomised control trial
RPa	Raphe pallidus nucleus
RR	Risk ratio
RYR1	Ryanoidine receptor, isoform 1
SAP	Shrimp alkaline phosphatase
SFO	Subfornical organ
SL	Sublingual
SNP	Single nucleotide polymorphism
SPPH	Severe PPH
SR	Sarcoplasmic reticulum
TBAS	Traditional birth attendances

T max	Time to peak concentration
TNF-α	Tumour necrosis factor alpha
TOP	Termination of pregnancy
T-Tubule	Transverse tubule
UTR	Un-translated regions
VBAC	Vaginal birth after caesarean section
VMPO	Ventromedical preoptic nucleus
WHO	World Health Organisation
x^2	Chi square test

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Abstract

Postpartum haemorrhage (PPH) is the main cause of maternal deaths worldwide. Despite the global effort to improve maternal health, developing countries are still far away from the proposed target of reducing maternal mortality. Therefore, a great deal of research is extensively going on globally to find out and improve appropriate treatment and technologies for low- resourced settings to prevent PPH and the work in this thesis on misoprostol was part of it.

Misoprostol is a uterotonic which can be given via different routes such as oral, rectal, vaginal and sublingual. Also, it is stable at ambient temperature and can be administered by non-skilled attendants. However, it has several adverse drug reactions but the increase in body temperature (fever) was the most common and irritating side effect. Therefore, reaching a balance between efficacy and safety was the overall aim of this thesis.

A systematic review was conducted to examine the incidence and the risk of fever after using misoprostol for prevention of PPH. Data showed that the occurrence of misoprostol induced fever was dose related and more with the sublingual route. Also, there was a variation in the incidence of fever in relation to the populations. To find out a low and effective dose of misoprostol with better adverse drug reaction profile, a randomised controlled trial was conducted to compare the effect of three different doses of sublingual misoprostol on the uterine activity. The choice of a suitable catheter to be used in this trial was done after conducting a validity study to compare two types of the catheters. The results of the trial showed that the three doses of misoprostol produced similar levels of uterine activity but the severity of fever was dose related. A genetic association with misoprostol induced fever was hypothesised as populations variations was documented in many RCTs and in the systematic review.

A pharmacogenetic study was carried out to examine the role of single nucleotides polymorphisms (SNPs) in two populations from Ecuador and Liverpool. There was an association between three SNPs in the prostaglandin transporters encoding genes and the increase in body temperature in the Ecuadorian population and this was not

reported in Liverpool population. Overall, while maintaining the efficacy of misoprostol, doses and routes can be optimised for the use in different populations to reduce the adverse drug reactions.

Chapter 1

Literature review

I. Misoprostol in obstetrics and gynaecology

II. Prostaglandin induced fever

III. Postpartum haemorrhage

Chapter 1. Literature review

This chapter will review the literature on misoprostol as a medication used for many obstetrics and gynaecological indications including the prevention and treatment of PPH. Finally, it will examine closely a common side effect of misoprostol: fever. First we will discuss drug-induced increases in body temperature in general and then highlight the mechanism and the molecular aspects of prostaglandin-induced fever and the possibility of investigating this issue by using pharmacogenetic approach. Also, it will explore the literature for up to date information about PPH including causes, prevention and treatment options

I. Misoprostol in obstetrics and gynaecology

1. Introduction

Misoprostol (15-deoxy-16-hydroxy-16-methyl PGE_1) was developed in 1973 as a stable synthetic form of prostaglandin E_1 analogue. It was originally developed for the treatment of gastric ulcers, using its anti-secretory properties. In addition, misoprostol has mucosal protective properties, which led to its use for the prevention of non-steroidal anti-inflammatory drug-induced peptic ulcers.

Prostaglandins have a potent effect on the female reproductive system. Misoprostol, being a prostaglandin analogue, is not different in this respect. It has become one of the most important drugs in obstetrics and gynaecology owing to its cervical ripening and uterotonic effects. However, its use in obstetrics and gynaecology is still off-label in many countries over the world and until recently, was not approved by the Food and Drug Administration (FDA) for use in pregnant women. In spring 2002, the FDA made a change to the misoprostol label: the statement that misoprostol is contraindicated in pregnancy was removed and a labour and delivery section was added.

In addition to the potent effect of misoprostol on uterine contractility and cervical ripening, misoprostol has the advantages of being cheap, widely available and stable at room temperature. This has made it suitable for use in developing countries and

became a central focus of obstetrics and gynaecology research over the past two decades.

This section will review the structure and chemistry of misoprostol, pharmacokinetics, uses, doses and side effects of misoprostol use in obstetrics and gynaecology.

2. Structure and chemistry of misoprostol

The rapid metabolic degradation of natural prostaglandins promoted the development of analogues suitable for clinical use. The most clinically important analogue is misoprostol. Its structure differs from the natural prostaglandin E by the presence of a methyl ester at C-1 and a methyl group and hydroxyl group at C-16 rather than at C-15. The addition of a methyl group at C-16 and the movement of the hydroxyl group from C-15 to C-16 improves oral activity and the safety profile of the drug as well as increasing its duration of action whereas, the presence of methyl ester group at C-1 increases the potency of its anti-secretory effect . Figure 1 shows the structure of natural prostaglandin E_1 and misoprostol.

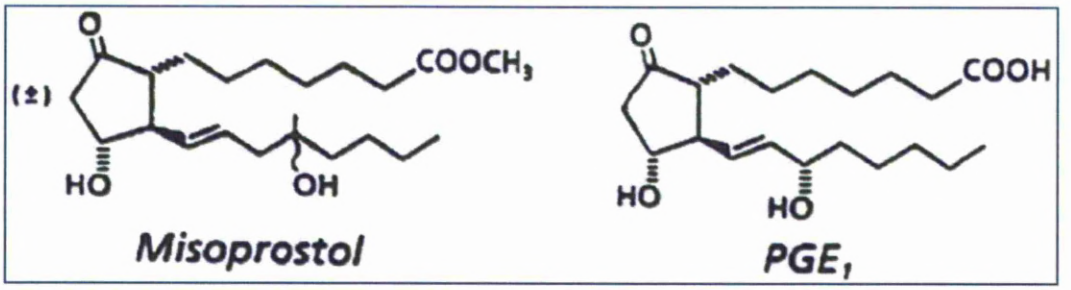

Figure 1. The structure of misoprostol and naturally occurring prostaglandin (PGE_1)

3. Misoprostol pharmacokinetics

Misoprostol is a prostaglandin E_1 analogue. It has a molecular weight of 382.54, exists as a mixture of two diastereomers and is stabilised by a dispersion on hydroxyl-propyl-methylcellulose (Karim 1987). Misoprostol is rapidly de-esterified to misoprostol acid, the biologically active metabolite. As a result of this rapid metabolic conversion, plasma concentrations of misoprostol are usually undetectable (Schoenhard, Oppermann & Kohn 1985). However, the absorption and metabolism of misoprostol has been studied using the 17, 18-tritium-labeled compound (Karim 1987). The absorption of misoprostol is rapid and extensive and most likely occurs in the stomach. Studies in many species including human found that the absorption of radioactivity takes place within 1.5 hours (Schoenhard, Oppermann & Kohn 1985). Plasma concentration of misoprostol acid reaches its peak in less than 30 minutes and its elimination half-life is 25-30 minutes after oral administration (Karim 1987).

Misoprostol acid is metabolised by beta oxidation to inactive dinor and tetranor metabolites (Figure 2, (Karim 1987) . Less than 1% of the dose is excreted in urine as unchanged misoprostol and its acid metabolite. Around 80% of the total radioactivity is excreted in the urine and faeces with in the first 24 hours of drug administration (Schoenhard, Oppermann & Kohn 1985). The presence of radioactivity in the faeces is attributed to the biliary excretion of misoprostol but not to the unabsorbed drug. This shows that at least 88% of the oral radio-labelled misoprostol is absorbed (Karim 1987).

The serum protein binding of radio-labelled misoprostol is 81-89% and was concentration-independent. There was no evidence that misoprostol is accumulated in red blood cells. Misoprostol has no effect on the hepatic mixed function oxidase (cytochrome P-450). Therefore, misoprostol does not affect the metabolism of other medicines that are metabolised by this enzyme system (Schoenhard, Oppermann & Kohn 1985). There was no accumulation in the plasma following multiple doses of misoprostol and steady state plasma concentrations were achieved within 2 days (Karim 1987).

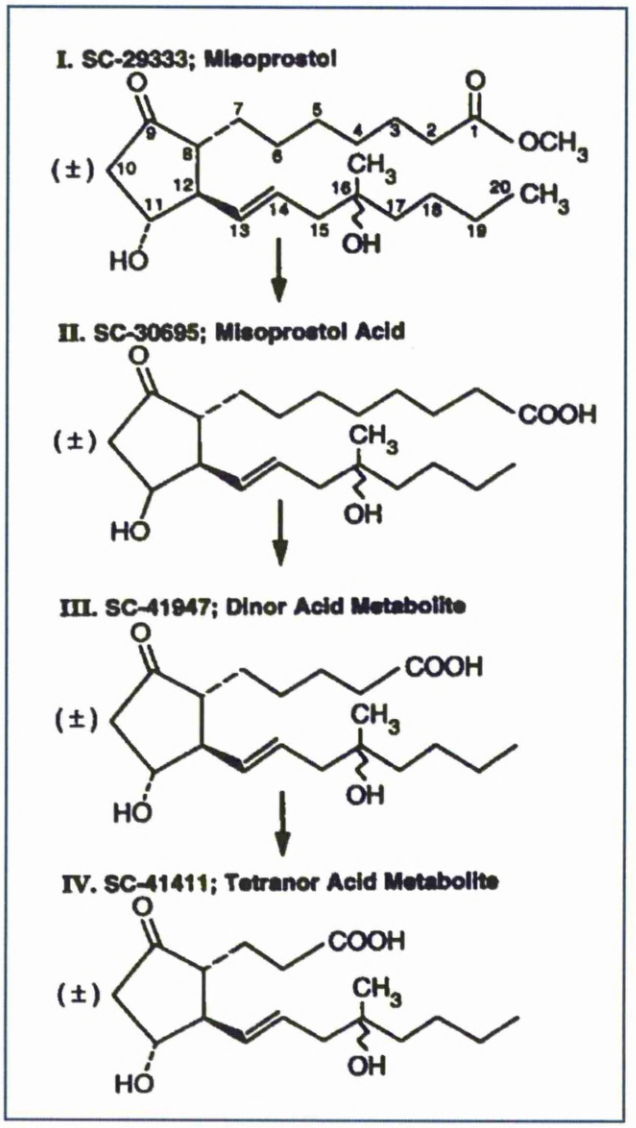

Figure 2. Structure of misoprostol (I), its biologically active misoprostol acid (II), and inactive dinor (III), and tetranor (IV) acid metabolites (From Karim A. 1987)

4. Pharmacokinetic profiles of various routes of administration of misoprostol

Studying and understanding the pharmacokinetics properties of different routes of administration of misoprostol can help to define the appropriate doses and design the best regimens for different clinical applications. Misoprostol is rapidly absorbed and undergoes extensive first-pass metabolism (de-esterification) to produce misoprostol acid which is active clinically and detectable in plasma. The peak concentration (C max), time to peak concentration (T max) and the area under the serum concentration versus time curve (AUC) are the three pharmacokinetic properties which have been studied. C max represents how well the drug is being absorbed; T max reflects how rapidly the drug can be absorbed whereas the AUC indicates the total exposure to the drug (the bioavailability). The peak plasma concentration of misoprostol acid is approximately 30 minutes after the oral ingestion of misoprostol tablets and decrease rapidly thereafter. Food or antacids ingestion with misoprostol decreases its bioavailability. Metabolism of misoprostol takes place first in the liver. It does not induce the hepatic cytochrome enzyme P-450 enzyme system (Goldberg, Greenberg & Darney 2001) and less than 1% of its active metabolite is excreted in urine (Foote et al., 1995).

Misoprostol is commercially available as an oral tablet in 100 mcg un-scored and 200 mcg scored tablets. However, other routes of administration including vaginal, rectal, buccal and sublingual have been extensively used for obstetrics and gynaecological indications.

4.1. Oral route

The pharmacokinetics properties of oral misoprostol have been studied and compared with other routes of administration. Following oral administration, misoprostol is rapidly absorbed from the gastrointestinal tract. After a single oral dose of 400 mcg misoprostol, the plasma misoprostol levels increase rapidly and reach the highest level at about 30 minutes, decline rapidly by 120 minutes and remain low thereafter (Khan et al., 2004; Meckstroth et al., 2006; Tang & Ho 2006; Tang et al., 2002); Figure 3).

Therefore, the oral route might be most appropriate when a rapid action is required as in cases of cervical dilatation prior to surgical procedures.

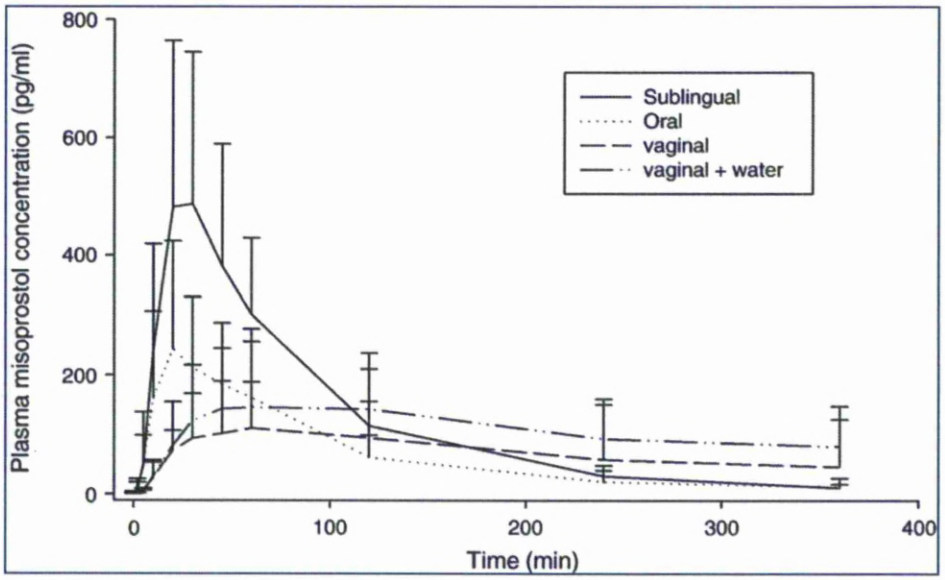

Figure 3. Mean plasma misoprostol acid concentration overtime after sublingual, oral, vaginal and moist vaginal misoprostol (Tang et al., 2002)

A slow –release (SR) form of oral misoprostol has been formulated (Chen et al., 2000). Higher doses of the SR form of misoprostol are needed to reach adequate serum levels to provide an effect on uterine contractility, while lower doses have almost no effect. 800 mcg of SR misoprostol has been studied and compared to 400 mcg of oral misoprostol (Fiala et al., 2005a). The SR form of misoprostol demonstrated a lower peak plasma level but a longer lasting elevated serum levels up to at least 12 hours in comparison to other routes of administration which suggested using SR misoprostol as an alternative to repeated administration of oral or vaginal misoprostol (Aronsson et al., 2007; Fiala et al., 2005a)(Figure 4).

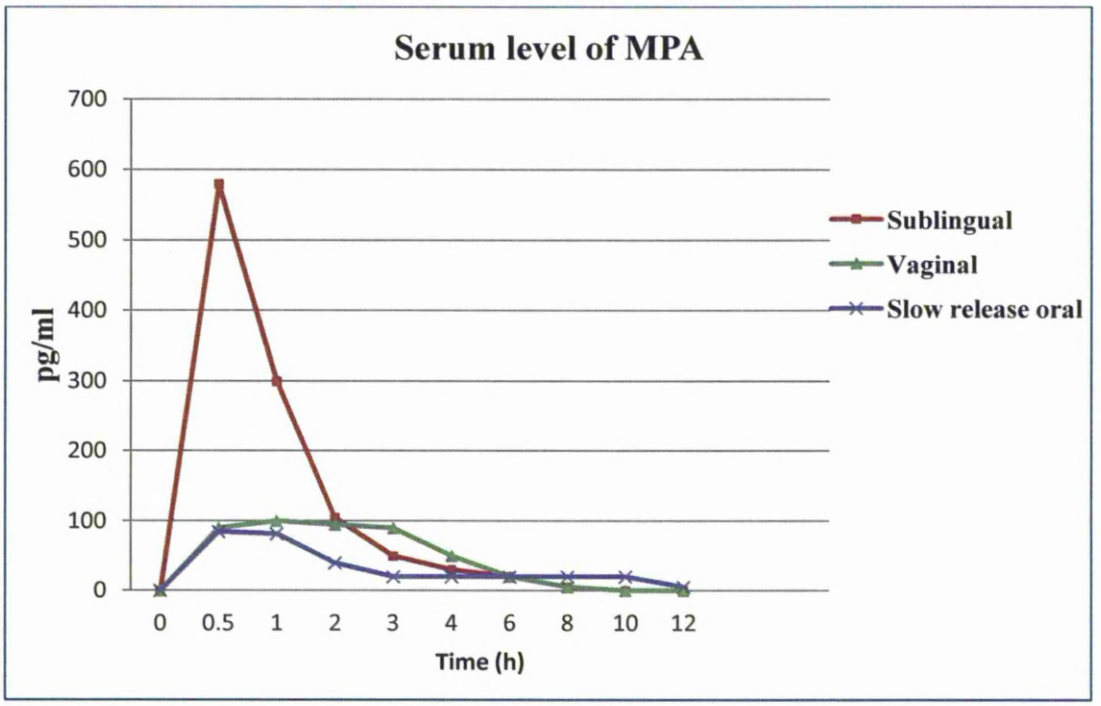

Figure 4. Mean Serum concentrations of MPA over time. (×) slow release;■ sublingual, ▲, vaginal. Reproduced from (Aronsson, A., et al., 2007)

4.2. Vaginal route

Although misoprostol tablets were originally developed for oral use, they are also effective when administered vaginally. After placing a tablet in the vagina, the plasma concentration increase gradually reaching a maximum level after 70-80 minutes (Figure 3). After this, it slowly declines with a still detectable drug level after 6 hours (Zieman et al., 1997). The bioavailability of vaginal misoprostol (area under the curve) is greater than for oral routes (Zieman et al., 1997) which might explain its effectiveness for stimulating uterine contraction, particularly for clinical indications where a longer duration of action is preferable, like medical abortion.

After vaginal administration it is not uncommon to find remnants of the tablets after some time. This indicates that absorption is variable and incomplete. Several attempts have been made to improve the absorption of vaginal misoprostol. Singh and his colleague used acetic acid to dissolve the tablets for women undergoing

termination of pregnancy, but it did not make a difference (Singh et al., 1999). The bioavailability of vaginal misoprostol was improved by adding water to the tablets and it was not significantly different from the sublingual route (Tang et al., 2002). On the other hand, progressive decline in the peak plasma level of misoprostol acid was observed in women with significant vaginal bleeding (Tang et al., 2009).

4.3. Sublingual route

The misoprostol tablet is very soluble when it is taken sublingually and is fully dissolved within 20 minutes. The time to peak concentration (T max) is about 30 minutes which is the same for oral administration but significantly shorter than following vaginal route of a administration which takes 75 minutes (Tang et al., 2002). The sublingual route has the highest serum peak concentration (C max) of misoprostol acid compared to vaginal, wet vaginal and oral routes. The sublingual route seems to have a quick onset of action with high serum concentration (C max). Hence, it might be more useful in cases where rapid onset of action is required, such as postpartum haemorrhage or cervical priming.

After administration of 400 mcg sublingual misoprostol, the achieved peak serum levels were significantly higher than those of oral and vaginal administration (Aronsson et al., 2007). This may be explained by the avoidance of the first –pass effect by the liver as well as the rapid absorption through the sublingual mucosa. This might be a result of abundant blood supply under the tongue and a relatively neutral pH (Tang et al., 2002).

The bioavailability of sublingual misoprostol at 6 and 12 hours, measured as (AUC_{360} and AUC_{720}) are significantly larger than that for vaginal or oral administration (Tang et al., 2002) (Aronsson et al., 2007). The AUC_{360} after oral and vaginal treatment are almost similar and constitute only 54% and 58% respectively of that after sublingual administration (Tang et al., 2002). On the other hand, Zieman et al found that the bioavailability is greater for vaginal misoprostol than for oral administration (Zieman et al., 1997). The differences in these studies' finding can be explained by the variation in the vaginal absorption among different women.

4.4. Buccal route

In this route of administration, misoprostol tablet is placed between the cheek and the teeth to be absorbed through the buccal mucosa. The tablets dissolve more rapidly in the sublingual compartment than in the buccal mucosa, it takes more than 30 minutes when administered buccally (Tang, Gemzell-Danielsson & Ho 2007). The T max is about 75 minutes after buccal administration which is similar to that after vaginal treatment. The serum concentration curve after buccal administration is similar to the vaginal route and is clearly different from the sublingual route (Figure 5) (Meckstroth et al., 2006). The structure of the buccal mucosa has more similarities to the vagina than the sublingual compartment, with a large surface area and thick epithelium. In contrast, the sublingual mucosa is thinner and more vascular (Meckstroth et al., 2006). Therefore, buccal administration of misoprostol can be an alternative to the vaginal route particularly in cases of leakage or profuse bleeding.

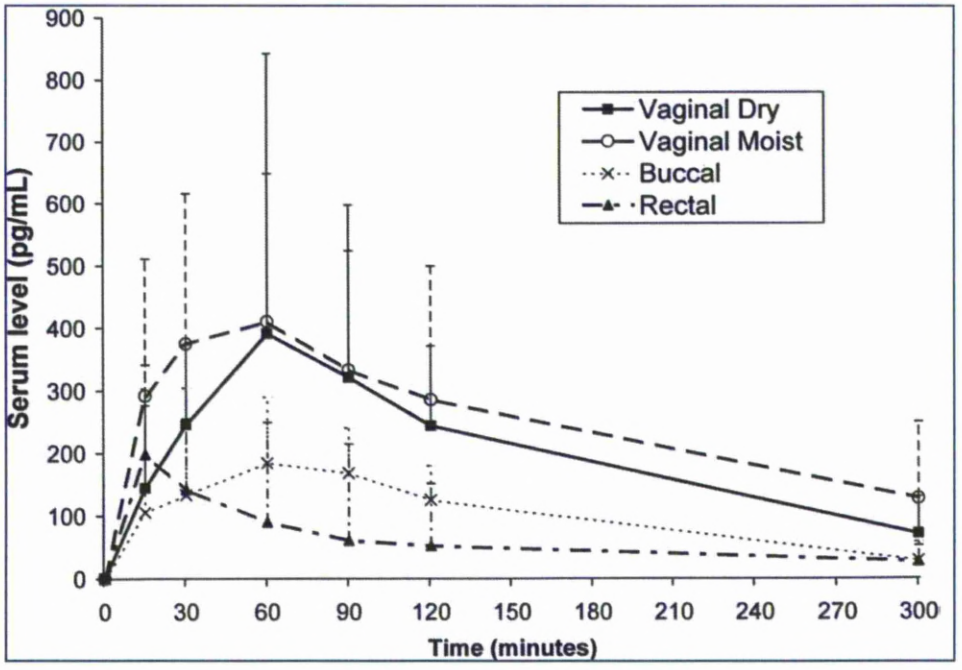

Figure 5. Mean serum levels of misoprostol acid in pg/mL for four epithelial routes of misoprostol administration over 5 hours. Error bars represent standard deviation (Meckstroth et al., 2006)

4.5. Rectal route

There are limited pharmacokinetics studies of rectal administration of misoprostol, and these give variable findings. Meckstroth and his colleagues found that serum misoprostol acid levels after rectal administration peaked at around 15 minutes, earlier than buccal and vaginal routes of administration, with rapid decline thereafter. The area under the curve (AUC) of misoprostol serum concentration after rectal treatment is only third of the vaginal route of treatment (Meckstroth et al., 2006). In contrast, Khan and his colleagues found that plasma concentration peaked at between 45-120 minutes (mean 71.7 minutes), then gradually declined. By 240 minutes, plasma concentration reached 46% of its maximum concentration. AUC_{240} of rectal misoprostol was not significantly different from the oral route whereas vaginal route AUC_{240} was the highest among the three routes (Khan et al., 2004), Figure 6).

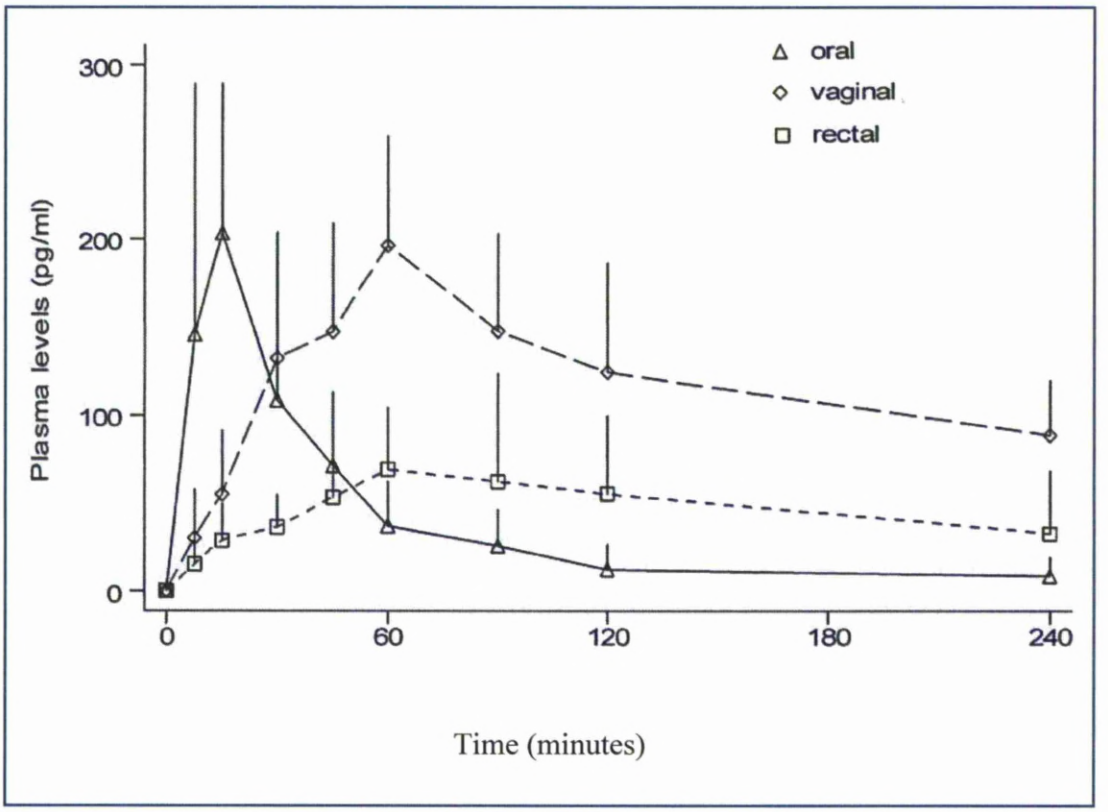

Figure 6. Mean plasma concentrations of misoprostol acid over time with oral, rectal, and vaginal administration. (Error bars represent 1 standard deviation) (Khan et al., 2004)

5. Effects of misoprostol on the uterus and the cervix

5.1. Effect on the uterus

Studies on uterine contractility and misoprostol have shown that a sustained level, rather than high serum level, is required to develop and maintain regular and effective uterine contractions. However, studies have failed to determine the threshold level for uterine contractility. This is complicated by the increase in uterine sensitivity to prostaglandins with increasing gestation (Tang, Gemzell-Danielsson & Ho 2007).

Studies show that a single oral dose of misoprostol can increase uterine tone. In order to produce uterine contractions, however, a high sustained plasma level of misoprostol is required and this needs repeated doses of oral misoprostol (Aronsson, Bygdeman & Gemzell-Danielsson 2004). In contrast, single dose of vaginal misoprostol can develop uterine tone due to its prolonged action. Regular uterine contractions develop after 1-2 hours and last for at least 4 hours after a single dose (Danielsson et al., 1999).

Larger doses of misoprostol will cause uterine tonus. This occurs more rapidly and more markedly after oral and sublingual misoprostol than following vaginal treatment. The mean time to maximum tonus is 8 and 11 minutes for oral and sublingual treatment respectively while after vaginal treatment, the maximum tonus is achieved after 20 minutes of administration. The uterine tonus ends after 1-2 hours of oral treatment while after vaginal administration it is slowly replaced with regular uterine contractions (Aronsson, Bygdeman & Gemzell-Danielsson 2004). This uterine activity is sustained for a longer time after vaginal treatment than after sublingual administration. The uterine contractions start to decrease after 4 hours of vaginal treatment compared to 3 hours following sublingual administration (Figure 7).

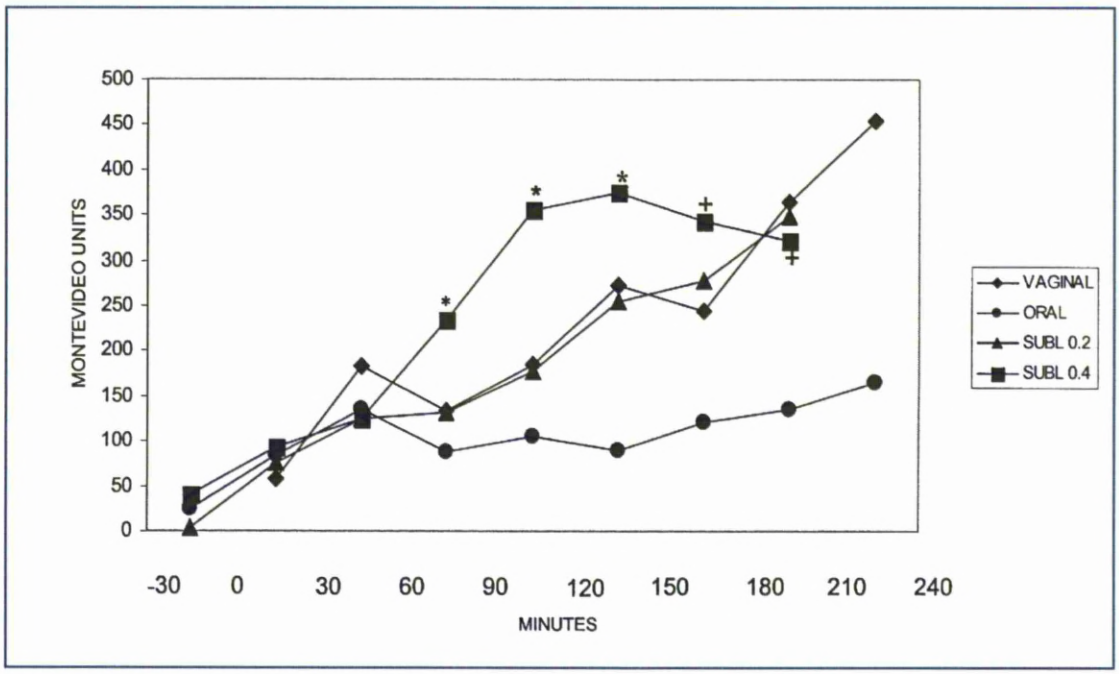

Figure 7. Uterine activity was measured in Mentevideo Units (MU). The treatment groups were as follows: Vaginal (0.4 mg), oral (0.4 mg) and sublingual (0.2 and 0.4 mg). Significant differences between the means of the sublingual (0.4 mg) and oral group: *P<0.05; (Aronsson, Bygdeman & Gemzell-Danielsson 2004).

The uterine effect of buccal and rectal misoprostol has been studied by (Meckstroth et al., 2006). The uterine activity after buccal administration is very similar to vaginal route, even though the bioavailability was two times less. Rectal misoprostol has got the lowest bioavailability, the lowest uterine activity and the longest mean onset of activity (103 minutes) among other routes of administration (Figure 8).

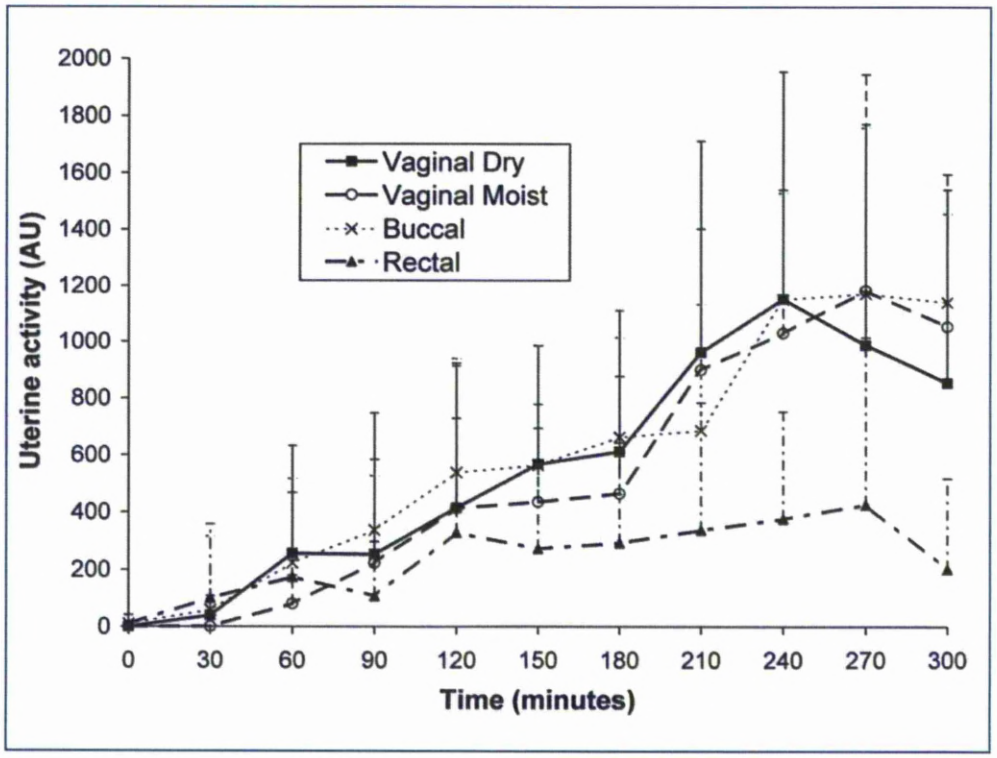

Figure 8. Mean uterine activity in Alexandria Units for four epithelial routes of misoprostol administration over five hours. Alexendria Units estimate area under the pressure across-time curve for uterine contractions. Error bars represent standard deviation. AU, Alexandria Units (Meckstroth et al., 2006)

5.2. Effect on the cervix

Misoprostol also causes softening of the cervix. It is likely that this is due to a direct effect on the cervix as well as secondary to the misoprostol induced uterine contractions. The uterine cervix is composed mainly of fibroblast cells and fibrous connective tissue which is mainly glycosaminoglycans and collagens. Ripening the cervix is an active and complex biochemical process in which collagen is degraded through an increase in its solubility and collagenolytic activity (Aronsson et al., 2005). Disintegration and dissolution of the cervical collagen fibre may be seen in cervical biopsies 2-4 hours after administration of 600 mcg vaginal misoprostol (El-Refaey et al., 1994). The mechanism of cervical softening is described as being similar to an inflammatory reaction. The influx of inflammatory cells to the cervical stroma increases matrix metalloproteinases which lead to degradation of collagen and softening of the cervix (Aronsson et al., 2005).

6. Misoprostol use in obstetrics and gynaecology

6.1. First trimester termination of pregnancy with misoprostol

In the last two decades, medical termination of pregnancy has become a safe alternative to surgical methods of termination in early pregnancy. Dilatation and curettage (D&C) was the only method used for safe abortion until the second half of the twentieth century, and in the 1960s, vacuum aspiration become the standard of care. Recently, however, the mifepristone / misoprostol regimen has become more widely available and considered to be the gold standard of early pregnancy termination (Faundes et al., 2007). A recent study shows that a single dose of vaginal misoprostol 800 mcg provides a similar success rate to surgical methods of termination. In addition, side effects were fewer with misoprostol and this medical method of termination was well accepted (Prasad, Kumar & Divya 2008). Many studies have been conducted on the use of misoprostol alone for medical abortion. However, a direct comparison of regimens is often not possible due to the wide variety of inclusion criteria and dosage regimens.

Many publications have shown the effectiveness of vaginal doses of 800 mcg of misoprostol repeated up to three times. This results in complete abortion in more

than 60% of cases (Blanchard et al., 2005). Oral misoprostol is less effective than vaginal (El-Refaey & Templeton 1994). Therefore, for termination of pregnancy, misoprostol should be administer vaginally unless there are reasons to avoid it (Blanchard et al., 2005). However, sublingual treatment is a good alternative and may be considered as a second choice.

A randomized control trial comparing vaginal and sublingual routes of misoprostol has shown that the intervals between vaginal misoprostol doses can be as long as 12 hours without significant effect on efficiency. On the other hand, sublingual misoprostol need more frequent administration, every 3 hours, to achieve a similar effectiveness to the vaginal route. However, it may cause more frequent gastrointestinal side effects, shivering and hyperthermia (von Hertzen et al., 2007). The recommended regimen is therefore 800 mcg of vaginal misoprostol administered every 6 hours for a maximum of three doses (Faundes et al., 2007).

6.2. Misoprostol to treat early fetal demise in the first trimester

The usual treatment of early fetal demise is suction curettage. However, several studies have shown that medical treatment is a safe, effective and acceptable alternative (Gronlund et al., 2002; Trinder et al., 2006; Weeks & Danielsson 2006). A study comparing 800 mcg of oral or vaginal misoprostol for treatment of early fetal loss showed that both methods were highly effective and acceptable. However, the mean time for expulsion was shorter in the vaginal group. It was 13.7 hours in the vaginal group versus 21.04 hours in the oral group (Ngoc et al., 2004). A study using a regimen of mifepristone in combination with misoprostol found that 800 mcg sublingual misoprostol and 800 mcg vaginal misoprostol were equally effective in inducing complete abortion, but a higher rate of side effects in the sublingual group (Tang et al., 2003). Lower doses of sublingual misoprostol have therefore been studied in an attempt to find an effective treatment with a low incidence of side effects. In a study of 50 women, a regimen of 600 mcg of sublingual misoprostol every 3 hours for a maximum of three doses was 86% effective for treatment of missed abortion (Sharma, Singhal & Rani 2007). Therefore, the recommended treatment of early fetal demise is 800 mcg vaginal misoprostol every 3 hours for a

maximum of 2 doses or 600 mcg of sublingual misoprostol every three hours repeated twice (Gemzell-Danielsson et al., 2007).

6.3. Treatment of incomplete miscarriage with misoprostol

The use of misoprostol to manage incomplete miscarriage could reduce the cost of health care through saving in surgical provider training, anaesthesia, sterilisation, hospitalisation and surgical equipment. Misoprostol can be provided at primary health care facilities for a low cost which, making it a good option in low-resource countries. Furthermore, the less invasive nature of misoprostol treatment makes it very acceptable to women worldwide (Blum et al., 2007b).

A regimen of a single dose of 600 mcg misoprostol has been compared with manual vacuum aspiration (MVA) for management of incomplete miscarriage. Both treatment options are effective with success rates of 91% to 96% respectively after 1-2 weeks of follow up (Bique et al., 2007; Weeks et al., 2005). However, misoprostol is more acceptable to women than MVA (Shwekerela et al., 2007). The use of double doses of 600 mcg of misoprostol increases the rate of side effects without clinical benefits (Phupong et al., 2004) (Blanchard et al., 2004).

There is now extensive evidence in the literature to support the use of misoprostol for treatment of incomplete abortion (Dao et al., 2007) . It has been suggested that it should now be considered the first line option for incomplete miscarriage (Gemzell-Danielsson, Fiala & Weeks 2007). The recommended regimen is a single dose of 600 mcg of oral misoprostol for women with incomplete miscarriage and uterine size equivalent to 12 weeks (Blum et al., 2007b).

6.4. Misoprostol for second trimester termination of pregnancy

Termination of early second trimester pregnancy can be performed by dilatation and curettage (D&C) or suction curettage, whereas termination performed late in second trimester requires cervical dilatation and fetal extraction or medical induction. Surgical procedures may result in severe complications include cervical laceration, uterine perforation and injuries to abdominal organs. Medical termination is potentially safer. Furthermore, it provides good histological specimens, a factor that is important if the termination is for fetal malformations. Medical termination is

therefore recommended by WHO (WHO 2003) and RCOG (RCOG 2004) using mifepristone followed by a prostaglandin analogue.

Several studies have investigated the use of misoprostol for second trimester induction of labour (Bhattacharyya et al., 2006; Carbonell et al., 2008; Dickinson & Evans 2002; Feldman et al., 2003; Herabutya, Chanrachakul & Punyavachira 2005; Pongsatha & Tongsong 2008; Tang et al., 2004; Wong et al., 2000). Women included in these studies have different duration of gestational age, received variable doses of misoprostol at different intervals and various route of administration. In addition to individual variations in women's drug response, the uterus becomes more sensitive to misoprostol with increasing gestational age and with fetal death (Srisomboon & Pongpisuttinun 1998). Therefore, these studies are difficult to compare.

Most of studies have used the vaginal route. However, the regimens were extremely variable. Carbonell and his colleagues found that 600 mcg of vaginal misoprostol given every 6 hours was as effective as 400 mcg of misoprostol every 4 hours with fetus mean expulsion time of 10.7 hours in the first group and 11.5 hours in the second group and the difference was not statistically significant (Carbonell et al., 2008). A study conducted by Khazardoost et al (Khazardoost, Hantoushzadeh & Madani 2007) concluded that 200 mcg of vaginal misoprostol every 6 hours up to 4 doses was as effective as 400 mcg misoprostol, and had less side effects. Even though women at earlier gestational age usually require larger doses for termination, women in this study responded well to as low doses as 200 mcg of misoprostol, even though they were all under 16 weeks.

Dickinson and his colleagues conducted a randomised trial of 3 regimens of misoprostol for intra uterine fetal death at 14-30 weeks. They found that 400 mcg vaginal misoprostol 6 hourly provided the optimal regimen, with a shorter delivery interval than 200 mcg dose and had fewer side effects than the 600 mcg dose (Dickinson & Evans 2002).

Even though uterine rupture is a serious complication of termination of pregnancy in the second trimester, several studies have reported safe use of misoprostol in women with scarred uterus (Rouzi 2003; Shammas & Momani 2006; Tarim et al., 2005). All

these studies recommended a careful consideration of doses and intervals for women with a scarred uterus. Further studies are essential before using misoprostol as a standard method for second trimester termination of pregnancy with a previous caesarean section.

There are a variety of successful regimens reported in the literature. In balancing efficacy and side effects, a regimen of 400 mcg vaginal misoprostol at 3 hours intervals for a maximum 5 doses is recommended (Ho et al., 2007). Higher doses may be needed for early second trimester abortion and lower doses may be sufficient to induce abortion later in the second trimester.

6.5. Misoprostol for intrauterine fetal death

There are a wide variety of clinically effective misoprostol regimens for induction of labour following second and third trimester IUFDs. The doses vary from 50 to 400 mcg given every 3 to 12 hours by different routes of administration (Chittacharoen, Herabutya & Punyavachira 2003; Fadalla, Mirghani & Adam 2004; Nakintu 2001; Pongsatha & Tongsong 2004). A recent study comparing vaginal, oral and sublingual misoprostol found that the induction to abortion interval was significantly shorter in the sublingual group compared to the oral and vaginal routes. The doses were 100 mcg given 4 hourly for the three routes of misoprostol (Elhassan, Abubaker & Adam 2008). Chittacharoen et al (Chittacharoen, Herabutya & Punyavachira 2003) reported that 400 mcg of oral misoprostol given 4 hourly is more effective than 200 mcg vaginal misoprostol administered every 12 hours, albeit with more side effects. On the other hand, many studies have recommended vaginal misoprostol for treatment of IUFD (Jain, Kuo & Mishell 1999; Khazardoost, Hantoushzadeh & Madani 2007; Srisomboon & Pongpisuttinun 1998). The required amount of misoprostol decreases with increasing gestational age and should be even less in women with IUFDs (Srisomboon & Pongpisuttinun 1998). Because of the risk of uterine rupture, it is recommended that women with scared uterus should receive lower doses of misoprostol and doubling of doses should not happen. Therefore, the recommended doses are variable according to the gestational age. From 13-17 weeks, 200 mcg vaginal misoprostol is required 6 hourly with 4 doses in maximum, 100 mcg vaginal misoprostol 6 hourly for a maximum of 4 doses is recommended for 18-26 weeks

and 25-50 mcg every 4 hours for a maximum of 6 doses is used for gestational age from 27-43 weeks. If the first dose does not produce effective contraction, the second dose could be doubled (Gomez Ponce de Leon, Wing & Fiala 2007).

6.6. Misoprostol for induction of labour with a live fetus

Induction of labour can be achieved by many medical and mechanical methods. Vaginal dinoprostone is the current gold standard method and is the drug of choice for cervical ripening and induction of labour (Weeks et al., 2007). Misoprostol has been used for cervical ripening and induction of labour since 1987. Several studies have been conducted to find out the best routes and dosages to reach equilibrium between high doses which cause uterine hyperstimulation and low doses which might lead to induction failure. Over 100 randomised trials have been reviewed for The Cochrane Library. In the review for vaginal misoprostol for induction of labour, Hofmeyr and Gülmezoglu (Hofmeyr & Gulmezoglu 2003) found that 25 mcg of vaginal misoprostol every 2 to 3 hours, 50 mcg every 4 hours and 100 mcg 6 to 12 hourly are all more effective than dinoprostone and oxytocin for induction of labour, but rates of uterine hyperstimulation increased with high doses. Meconium stained liquor is also significantly associated with misoprostol usage. Whether it is due to uterine hyperstimulation or due to the direct effect of misoprostol on fetal bowel is still not proven.

Alfirevic and Weeks reviewed 41 trials to assess the effectiveness and safety of oral misoprostol for induction of labour with viable fetus. In comparison of vaginal misoprostol, 50 mcg oral misoprostol every 4 to 6 hours seems to decrease the need for caesarean section in women with intact membrane. With no significant difference in uterine hyperstimulation and meconium stained liquor rates. The authors recommended a maximum of 50 mcg of oral misoprostol for induction of labour (Alfirevic & Weeks 2006).

Titrated oral misoprostol can be made by dissolving one tablet (200 mcg) into 200 ml of tap water. Hofmeyr and his colleagues (Hofmeyr et al., 2001a) used 20 mcg of misoprostol every 2 hours while Cheng his colleagues (Cheng, Ming & Lee 2008) used 20 mcg every 1 hour for 4 doses and adjusted it against individual uterine response. This regimen has associated with low incidence of uterine hyperstimulation

and low caesarean section rate as well as shorter induction to labour interval than vaginal misoprostol. Therefore, 20 mcg of oral misoprostol solution every 2 hours for a maximum of 12 doses has been recommended (Weeks et al., 2007). A systematic review of 9 studies with 2937 women concluded that low-dose oral misoprostol solution (20 mcg) administered every 2 hours seems at least as effective as both vaginal dinoprostone and vaginal misoprostol, with lower rates of caesarean delivery and uterine hyperstimulation, respectively (Kundodyiwa, Alfirevic & Weeks 2009).

Few studies have conducted to investigate the effect of buccal and sublingual misoprostol for induction of labour. More research is required to establish the effectiveness, safety and optimum dosage of buccal and sublingual misoprostol for induction and cervical ripening (Muzonzini & Hofmeyr 2004).

Women who have a history of a previous uterine scar are at increased risk of ruptured uterus when induced by misoprostol (Wing, Lovett & Paul 1998). The risk of uterine rupture with misoprostol was increased in women who attempting vaginal birth after caesarean section (VBAC) and who have not had a previous vaginal delivery (Smith et al., 2004). A trial of vaginal delivery after previous caesarean section has shown that uterine rupture occurred in 5.6% of patients who induced by misoprostol which is significantly higher when compared to 0.2% in those who did not (Plaut, Schwartz & Lubarsky 1999). On the other hand, one study reported no uterine rupture in 48 women who had a history of caesarean section and were given misoprostol for induction (Choy-Hee & Raynor 2001). However, there is no enough evidence for the safety of misoprostol for induction with previous uterine scare (Plaut, Schwartz & Lubarsky 1999). Therefore, the use of misoprostol is contraindicated in this situation.

The recommended doses for induction of labour are 25 mcg vaginal misoprostol or 50 mcg of oral misoprostol every 4 hours for a maximum of 6 doses. Another option is 20 mcg of oral misoprostol solution 2 hourly for a maximum of 12 doses (Weeks et al., 2007).

6.7. Cervical priming with misoprostol before transcervical procedures

Cervical priming means softening or dilating the cervix before transcervical procedures including surgical abortion, hysteroscopy, dilatation and curettage, insertion of intrauterine device and endometrial biopsy. Cervical priming can facilitate mechanical dilatation, shorten the operation time, reduce blood loss and decrease the frequency of complications (Fiala et al., 2007) and can be achieved medically by prostaglandins analogues. Administration of misoprostol prior to transcervical procedures results in clinical and histochemical changes which involves the influx of inflammatory cells into the stroma. This degrades of the collagen leading to softening and dilatation of the cervix (El-Refaey et al., 1994).

Misoprostol has been used effectively for cervical priming in pregnant women. Studies show that both oral and vaginal misoprostol are useful for cervical priming prior to vacuum aspiration (Ngai et al., 1999). The dose of 400 mcg of vaginal misoprostol administrated 3 hours before the procedures is recommended as an optimal dose (Fong, Singh & Prasad 1998). The same regimens using either oral or sublingual misoprostol were as effective as the vaginal route (Ngai et al., 1999; Tang, Mok & Ho 2004). Another study found that the vaginal route is more effective than oral misoprostol (MacIsaac et al., 1999). 200 mcg sublingual misoprostol administered 2 hours before suction curettage was as effective as 400 mcg vaginal misoprostol 3 hours before that procedure with higher pain scores and similar patients' satisfaction (Caliskan et al., 2007). However, oral and sublingual routes have the advantages of being more convenient and more acceptable to women (Chong, Chua & Arulkumaran 1997; Tang, Mok & Ho 2004).

Misoprostol can also be used for cervical priming in non-pregnant women. Ngai and his colleagues investigated the effect of 400 mcg oral misoprostol given 12 hours before the procedure on the cervix prior to hysteroscopy. Data showed that misoprostol was more effective than placebo in terms of significantly reducing the amount of force required for cervical dilatation and increasing the mean baseline cervical dilatation from 3.3 mm in the placebo group to 6.0 mm in the misoprostol group (Ngai et al., 1997). Misoprostol is also effective in cervical softening in nulliparous women. Administration of 400 mcg of sublingual misoprostol has

facilitated the insertion of intrauterine device in those women. The most common side effect associated with this regimen was shivering which could be reduced by using oral and vaginal routes (Saav et al., 2007).

Misoprostol has shown considerable effect on the uterine cervix in the premenopausal women. One thousand micrograms of vaginally self administered misoprostol the evening prior to operative hysteroscopy has shown a significant effect on cervical ripening in pre-menopausal women but no effect on the postmenopausal women. However, this dose was associated with lower abdominal pain and slight preoperative bleeding (Oppegaard et al., 2007). Batukan et al investigated a dose of 400 mcg vaginal versus 400 mcg oral misoprostol in premeopausal women for cervical priming prior to operative hysterescopy. The vaginal route was more effective than the oral misoprostol in increasing the mean cervical width, shortening the time required for cervical dilatation and the duration of surgery. However, the vaginal misoprostol was associated with excessive fluid leakage and cervical softening (Batukan et al., 2008).

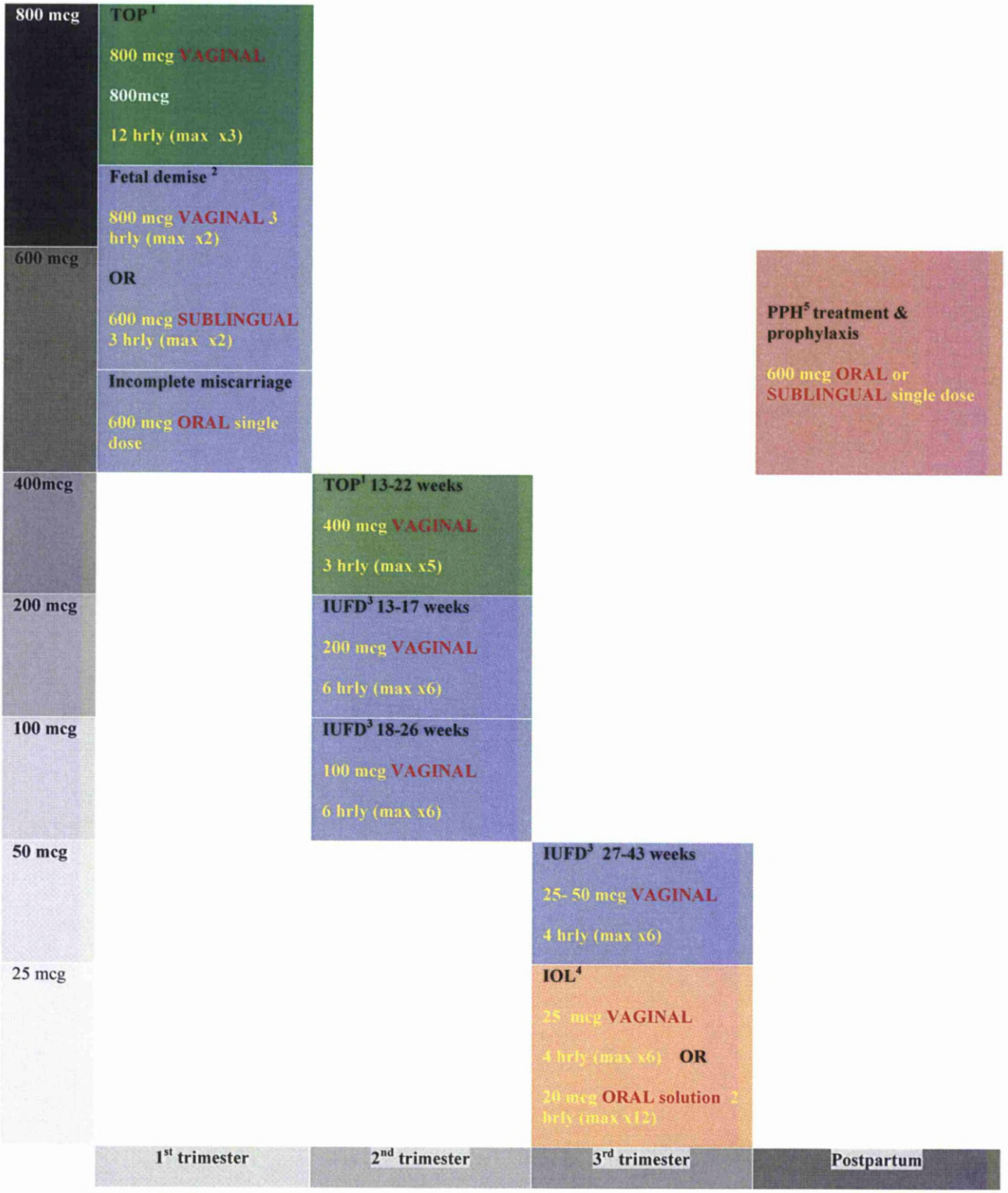

Figure 9. Recommended doses of misoprostol. Recommendations from: Weeks, A. & Faundes, A. Misoprostol in obstetrics and gynaecology. Int J Gynaecol Obstet, 2007. 99 Suppl 2: p. S156-9 (1. Termination of pregnancy or medical abortion; 2. Also known as missed or silent miscarriage; 3. Intrauterine fetal death; 4. Induction of labour with a live fetus; 5. Postpartum haemorrhage.

6.8. Prevention of postpartum haemorrhage with misoprostol

PPH is the most preventable cause of maternal death. Providing cheap, stable and effective therapy for prevention of PPH is essential for reducing maternal mortality in low-resource countries. Several studies have now demonstrated the efficacy of misoprostol in preventing PPH. An initial trial comparing 500 mcg of oral misoprostol to standard oxytotic regimen suggested that oral misoprostol was comparable to oxytotic drugs for prevention of postpartum haemorrhage (El-Refaey et al., 2000). Another multicenter randomised controlled trial including 2058 women and comparing 600 mcg of oral misoprostol to 1 ml intramuscular syntometrine found that misoprostol is comparable to syntometrine in the incidence of PPH, the mean blood loss and the fall in haemoglobin concentration (Ng et al., 2001). However, a much larger WHO multicentre randomised trial showed that misoprostol is worse than oxytocin in hospital settings. This study showed a significant difference in the amount of blood loss and the need of additional uterotonics. In addition, misoprostol was associated with a significantly higher incidence of shivering and fever (Gulmezoglu et al., 2001). Comparison of misoprostol with a placebo in rural settings shows that misoprostol is very effective in prevention of PPH.

A prospective single-blinded, randomised controlled trial was conducted in Nigeria in a quite poor standard care hospital where cold storage facilities are below standard, which compared to most rural health centres in Nigeria. 864 women received either 400 mcg of oral misoprostol or 500 mcg of intramuscular methylergometrine. Those given oral misoprostol had significantly lower rates of PPH although prolonged third stage of labour (>15 min) and need for manual removal of placenta were more in the misoprostol group (Enakpene et al., 2007). This result could be explained by poor cold storage for the methylergometrine. Therefore, misoprostol could be a good alternative to methylergometrine in poor resource setting. A randomised double blind placebo controlled trial; undertaken in 661 women in primary health centre in Guinea-Bissau showed that 600 mcg sublingual misoprostol reduced the frequency of severe PPH $\geq$ 1000 ml; (Hoj et al., 2005). Another placebo randomised controlled trial included 1620 women in primary health centres in rural India and found that 600 mcg oral misoprostol reduced acute and severe bleeding postpartum by 50% compared

with placebo (Derman et al., 2006). A trial conducted in 26 villages in rural Gambia with 52 traditional birth attendants (TBAs) to compare 600 mcg of oral misoprostol to placebo and 4 tablets of 0.5 mg ergometrine found that misoprostol group had lower incidence of blood loss and mean drop in Hb was significantly lower in the misoprostol group (Walraven et al., 2005).

To determine the feasibility of implementing community based distribution of misoprostol for prevention of PPH within the government health system, a recent study was conducted in a remote setting in Nepal. This study showed that the overall coverage of uterotonics among women with vaginal birth increased from 11.6% to 74.2% (OR 25.0, 95% CI 15.6–40.1). Regarding the safety, none of the women reported taking it before the delivery and the maternal mortality rate was lower in the women who used misoprostol (10 deaths among 13,969, giving a mortality ratio of 72 per 100,000) compared to the estimated national rate of 281 per 100,000 (95% CI 178–384) and to the non-users of misoprostol (292 per 100,000; i.e. 35 deaths among 12,031 births; (Rajbhandari et al., 2010). A systematic review of randomised controlled trials with a total of 28,138 women to assess the effects of prophylactic misoprostol in the third stage of labour concluded that injectable uterotonic drugs are more effective than misoprostol (Villar et al., 2002). A recent Cochrane systematic review comparing prostaglandins for prevention of PPH concluded that a dose of 600 mcg oral or sublingual misoprostol shows promising results in reducing blood loss postpartum compared to placebo. The authors recommended further research to find out the optimal route with a minimum effective dose of misoprostol for routine use for prevention of PPH to reduce the side effects which are dose related (Gulmezoglu et al., 2007). Based on the above evidence the WHO recommended the use of oxytocin rather than 600 mcg of oral misoprostol for prevention of PPH (WHO 2007). In addition, expert group recommended single doses of 600 mcg of oral or sublingual misoprostol for the prevention of PPH when injectable uterotonic drugs are not available and this dose should not be repeated for 2 hours (Weeks & Faundes 2007).

6.9. Treatment of postpartum haemorrhage with misoprostol

The treatment of PPH is based mainly on the administration of uterotonic drugs, with parenteral oxytocin and ergometrine being the most commonly used agents. However, they are not widely available, require refrigeration and relatively expensive (Blum et al., 2007a). Small observational study shows that misoprostol is highly effective in treatment of PPH. This study conducted to assess the efficacy of rectal misoprostol as a second line treatment of primary PPH by comparing it to methylergonovine maleate. The doses of misoprostol were ranging from 800 to 1000 mcg. This results showed no significant difference between the two groups in the need for blood transfusion, the need for third line medical therapy and surgical intervention (Baruah & Cohn 2008). However, this study has some limitations including small sample size and it was a retrospective one and subjected to selection bias.

Two small randomised controlled trials found that misoprostol is associated with reduction in blood loss and recommended that misoprostol should be available to women who do not have access to the conventional uterotonic drugs, oxytocin and ergometrine (Hofmeyr et al., 2004; Walraven et al., 2004).

The need for more stable, less expensive and easily administered drugs has turned the attention toward misoprostol and therefore, parachute approach were used in some countries to implement policies based on good science without randomised controlled trials especially in poor setting with high maternal mortality, when a simple intervention can save lives (Potts et al., 2006). Nigeria was the first who approves misoprostol for treatment of PPH (Jadesimi & Okonofua 2006) followed by Uganda and Ethiopia . This happened as a result of increasing evidence supporting its use in low resource sitting, where oxytotic drugs are not available.

Since the Cochrane review of treatment of PPH which was published in 2007 and updated in 2009, 3 large multi-centre double blind, randomised trials were published in the Lancet (2010). These trials investigated and compared the misoprostol to oxytocin in three different scenarios. Their data provided strong evidence that misoprostol is inferior to oxytocin when used after physiological management of the third stage of labour and equivalent to oxytocin after AMTSL. Also, the data showed

that misoprostol was not as effective as placebo when used as an adjunct to standard uterotonics for treatment of PPH.

The first trial compared 800 mcg sublingual misoprostol to 40 IU intravenous oxytocin for the treatment of PPH in women who had not exposed to oxytocin in labour. The study involved 9348 subjects from three different populations in Ecuador, Egypt and Vietnam. 978 (10%) of them were diagnosed with PPH and received the study treatments. The results showed that the cessation of active bleeding within 20 minutes was achieved in 440 out of 488 women who received misoprostol (90%) and in 468 out of 490 women who was treated by oxytocin (96%) and (RR 1.78, 95% CI 0.91-0.98). Also, additional blood loss of $\geq$ 300 ml after treatment occurred in 30% of the women who managed with misoprostol and in 17% in women who received oxytocin (RR 1.78, 95% CI (1.40-2.26), whilist additional loss of 1000 ml after treatment occurred in 1 or less of the women in the both study arms (RR 1,67, 95% CI 1.40-6.96, P=0.36). Although data provided evidence that oxytocin is significantly better than misoprostol for PPH treatment, the authors still recommended the use of misoprostol as a first line treatment of PPH in setting where oxytocin is not feasible (Winikoff et al., 2010).

The second trial examined and compared the 800 mcg sublingual misoprostol 40 IU oxytocin given in a litre of intravenous solution over 15 minutes for the treatment of PPH after AMTSL with oxytocin. This trial included 31055 women and 809 (3%) were diagnosed with PPH after exposed to prophylactic oxytocin. The active bleeding was controlled within 20 minutes in 363 (89%) women managed with misoprostol and in 360 (90%) of women given oxytocin [RR 0.99, 95% CI (0.95-1.04)]. The incidence of additional blood loss of $\geq$ 300 ml after treatment was higher in the misoprostol group (34%) vs. (31%) in the oxytocin group (RR 1.12, 95% CI 0.92-1.37), whilst blood loss > 1000mls after treatment occurred in 11 (3%) women managed with misoprostol and in 3 (1%) of women given oxytocin (RR 3.62, 95% CI 1.02-12.86). These finding suggested that 800 mcg sublingual misoprostol is a possible alternative to 40 IU intravenous oxytocin for the management of PPH after AMTSL. However, misoprostol administration was significant associated with more side effects than with

oxytocin. The incidence of shivering were (37% vs. 15%) and fever were (22% vs. 15%) for misoprostol and oxytocin respectively (Blum et al., 2010) .

The third study explored the possibility of using misoprostol in addition to conventional injectable uterotonics to treat PPH. The study compared 600 mcg sublingual misoprostol with placebo. The finding showed no significant difference between the two treatment groups in the proportion of blood loss of ≥ 500 ml within 60 minutes [100/705 (14%) vs. 100/717 (14%)]. As the other two trials, the incidence of adverse drug reactions were more in the misoprostol group compared to no treatment. Therefore, this study does not support the use of misoprostol in addition to other uterotonics for the treatment of PPH. The rate of shivering was 65% and 32% and the frequency of fever ≥ 38°C was 43% and 15% respectively (Widmer et al., 2010).

This strong evidence clearly showed that misoprostol is inferior to oxytocin for the treatment of PPH when used after physiological management of the third stage of labour and it was equivalent to oxytocin after AMTSL. Also, evidence showed that misoprostol was as effective as placebo in conjunction to standard uterotonics for treatment of PPH. Therefore, these trials confirmed that oxytocin is still the first line for the treatment of PPH in hospital sitting and where it is available and misoprostol is the first choice of treatment where injectable uterotonics are not available particularly in poor and remote areas.

7. Cost-effectiveness of misoprostol for prevention and treatment of PPH

One of the main targets to help reducing maternal mortality in the developing world is producing an affordable medicine for the management of PPH. An observational study that traditional birth attencence (TBAs) administered 1000 mcg of misoprostol rectally when PPH occurred helped to prevent 1647 cases of severe PPH. It was estimated that this saved $ 115.335 in the cost of referral, IV therapy and transfusions per 10,000 births (Bradley et al., 2007). The cost-effectiveness of 600 mcg of misoprostol for the prevention of PPH and reducing maternal mortality was investigated in an observational study conducted in rural India. Administration of

misoprostol by TBAs after the delivery resulted in a 38% (95% CI 5%-73%) decrease in the maternal deaths. The cost effectiveness estimate of US$ 1401 for each life saved was favourably compared to other public health intervention. For instant, the cost-effectiveness of improving comprehensive emergency obstetric care per life saved is US$10,532 (Sutherland & Bishai 2009), which is more expensive than misoprostol and unaffordable to the developing counties with the highest rates of maternal mortalities.

A recent study modelled the cost-effectiveness of standard management plus 800 mcg sublingual misoprostol for treatment of PPH and the standard management plus 600 mcg oral misoprostol for prevention of PPH versus the standard management alone in rural India. For example, in one region with 3,888,000 home births and with an MMR of 440, the total cost of the misoprostol treatment package would have been $ 1,644,181. Each year, they estimate that this intervention would save the lives of 2994 women who would have died because of PPH (Sutherland et al., 2010) making a cost of around $ 550 per life saved. Large scale implementation of misoprostol for prevention and treatment of PPH requires careful consideration of cost, efficacy and safety in the low resource settings where illiteracy and poverty are common.

8. Side effects of misoprostol

8.1. Chills and /or fever

Chills are fairly common in association with the use of misoprostol but are transient. Hyperthermia can be severe (Chong, Chua & Arulkumaran 1997) with high doses, shorter intervals and particularly with oral and sublingual routes. (More details in Chapter 4, the systematic review of misoprostol induced fever).

8.2. Gastrointestinal side effects

Nausea, vomiting and diarrhoea are quite common adverse reactions of misoprostol intake. About 35% of women will experience gastrointestinal side effects (Gomez Ponce de Leon, Wing & Fiala 2007). If there is significant vomiting, anti-emetic can be offered and it usually resolves in 2 to 6 hours. Diarrhoea is the major side effect and it is usually mild and self-limiting within a day. The gastrointestinal side effects

are more common with oral and sublingual administration (Tang, Gemzell-Danielsson & Ho 2007).

8.3. Uterine hyperstimulation and rupture

Hyperstimulation may occur as a result of using too high doses as well as with the recommended dosage of misoprostol. Several studies show a greater incidence of uterine hypercontractility with misoprostol compared with oxytocin. The incidence of hyperstimulation with misoprostol is 4-12% which is similar to the rate after induction with dinoprostone (Alfirevic & Weeks 2006; Hofmeyr & Gulmezoglu 2003).

Uterine rupture is another concern with the use of misoprostol. However, it is rare in the first trimester termination of pregnancy. Kim and his colleagues have reported a case of uterine rupture with misoprostol at 8 weeks gestation (Kim et al., 2005). The most cases of uterine rupture took place during induction of labour in the third trimester and associated with a previous uterine scar (Plaut, Schwartz & Lubarsky 1999).

8.4. Abdominal cramps

Abdominal cramps usually develop with in the first few hours and may start as early as 10 minutes. The pain can be stronger than that of a regular period. NSAIDs can be given for pain relief without affecting the effectiveness of misoprostol (Fiala et al., 2005b). More analgesia is required in younger women, with advanced pregnancies and with multiple doses of misoprostol whereas women with previous live birth were significantly less likely to require analgesia (Hamoda et al., 2004).

8.5. Fetal abnormalities

The risk of fetal malformation after use of misoprostol is low. The estimated risk is about 1% among exposed fetus (Tang, Gemzell-Danielsson & Ho 2007). Multiple congenital abnormalities have been reported after unsuccessful use of misoprostol for termination. The association between misoprostol at abortifacient doses and some congenital malformation might indicate a real teratogenic effect of misoprostol (Orioli & Castilla 2000). The association between Mobius syndrome (congenital facial

paralysis with or without limb defects) and exposure to misoprostol has been reported by Pastuszak et al (Pastuszak et al., 1998).

8.6. Skin rash

Administration of misoprostol occasionally leads to development of skin rash which resolve within several hours (Blum et al., 2007b).

Table 1. Systemic, local and teratogenic adverse drug reaction for misoprostol

Systemic	Gastrointestina; side effects (nausea, vomiting, diaahoea) Abdominal cramps Chills and/ or fever	Incidence: 35% (Gomez Ponce de Leon, Wing & Fiala 2007)
Local	Uterine hyperstimulation and rupture	Incidence: 4-12% (Hofmeyr & Gulmezoglu 2003)
Teratogeneic	Multiple congenital abnormalities Mobius syndrome (congenital facial paralysis with or without limb defect)	Estimated risk 1% (Tang, Gemzell-Danielsson & Ho 2007)

II. Prostaglandin-induced fever

1. Introduction

Misoprostol, which is a prostaglandin analogue, has similar side effects to prostaglandins, which were mentioned in the previous section. The most common side effect is pyrexia (fever). Although drug-induced increase in body temperature (such as malignant hyperthermia) has been extensively reported and examined in the literature, the mechanism of misoprostol-induced fever has not yet been studied. Therefore, this section will discuss prostaglandins-induced fever in terms of its mechanism and its molecular aspects. This section will also give an overview of pharmacogenetics and adverse drug reactions.

2. Fever, hyperthermia and hyperpyrexia

Although the terms hyperthermia (un-regulated elevation of body temperature) and hyperpyrexia (fever with an extreme elevation of body temperature, >41.5°C) are sometimes used synonymously, they are actually different in terms of both mechanism and treatment. Hyperpyrexia results when a pathogen, insult or drug cause the hypothalamus to increase its core temperature set-point in a regulated manner, whereas hyperthermia is a process by which stress, extreme heat and drugs increase the body's metabolic state, increase heat production and/or decrease heat loss without impairment of the temperature set-point in the hypothalamus. This results in un-regulated increase in body temperature, making it particularly dangerous (Howard 1993). Therefore, antipyretics can be used to treat fever by reducing the elevated set-point in the hypothalamus (Hadad, Weinbroum & Ben-Abraham 2003). During hyperthermia, skin blood flow increases which in turn, leads to heat loss through the evaporation of water (sweating). In contrast, with hyperpyrexia, febrile people do not sweat but rather shiver to increase metabolic heat production to the new set-point in the hypothalamus (Roth et al., 2006). The existence of a common mediator of fever was postulated as it can be produced by many types of administered substances peripherally ranging from organic and inorganic compounds. and microbial and mammalian proteins (Conti et al., 2004).

3. Aetiology of increased body temperature

Increased body temperature or fever is the most common clinical symptom and can be a presentation in a vast array of illnesses and conditions. Table 2 summarises some of the causes of increased body temperature (Laupland 2009).

Table 2. Most common causes of fever (adapted from Laupland 2009)

Infectious diseases (bacterial, fungal, viral and protozoa)	Influenza, malaria, measles, infectious mononucleosis, typhoid fever, yellow fever
Neoplastic diseases	Leukaemia, lymphomas, kidney cancer
Rheumatic diseases	Rheumatic arthritis, rheumatic fever
Immunological and collagen diseases	Sarcoidosis, lupus erythematosus
Metabolic and inherited diseases	Gout, prophyria, Familial Mediterranean fever
Hematologic causes	Transfusion reactions, pulmonary embolism, deep vein thrombosis
Inflammations	Boils, abscess, wound infection
Tissue destruction	Crush syndrome, infarction, haemolysis, surgery
Environmental causes	Heatstroke
Drug-induced fever	Succinylcholine, cocaine, amphetamine, misoprostol
Endocrine diseases	Thyrotoxicosis, pheochromocytoma, and adrenal crisis
Fever of unknown origin	

4. Molecular aspects of drug-induced increase in body temperature

Drugs can increase body temperature through several mechanisms. Some drugs can cause hyperthermia such as malignant hyperthermia, produced by succinylcholine and some drugs may cause hyperpyrexia, such as prostaglandin-induced fever. The mechanisms of drug-induced increase in body temperature, however, are multifactorial and incompletely understood. They are mainly classified into five categories: hypersensitivity reactions, directly drug-induced, altered thermoregulation, direct drug's pharmacological action and idiosyncratic reactions (Johnson & Cunha 1996).

Thermoregulation is simply the balance between heat loss and heat gain. It uses both central and peripheral mechanisms to maintain the body's temperature within tightly controlled limits. Severe and fatal conditions can result from failure of the thermoregulation system. There are various mechanisms of drug-induced fever which alter the balance between heat production and heat loss, involving many structures, from the hypothalamus down to the brain stem and spinal cord (Boulant 2000). The preoptic region neurons near the rostral hypothalamus are very sensitive to changes in core temperature and receive inputs from the skin and spinal chemoreceptors. Both central and peripheral information are integrated in the preoptic region to adjust the core temperature at a pre-defined set-point. This is achieved by regulating regional and cutaneous blood flow, hormonal response, sweating and shivering (Hadad, Weinbroum & Ben-Abraham 2003). The mechanism of hyperthermia may result from increased basal metabolic activity, absorption of heat from the environment and increased muscle activity. In addition, the preoptic region communicates with lower brain stem areas via the median forebrain bundle pathway passing through the lateral hypothalamus. This pathway for preoptic efferent signals control skin blood flow (Kanosue, Yanase-Fujiwara & Hosono 1994) and shivering (Kanosue et al., 1994). This section shows the difference between these two types of drug-induced increase in the body temperature, including the mechanism, the molecular and the genetic aspects.

4.1. Molecular aspects of drug-induced hyperthermia

Drug-induced hyperthermia is defined as hyperthermia which develops following drug administration and which disappears after cessation of drug administration, providing that no other causes of fever are evident (Mackowiak & LeMaistre 1987). It is mainly diagnosed by exclusion. There are five well-known syndromes of drug-induced hyperthermia: malignant hyperthermia, anticholinergic poisoning, serotonin syndrome, neuroleptic malignant syndrome, and sympathomimetics poisoning (Table 3). The symptoms can vary from simple elevation in body temperature to life-threatening conditions (Hadad, Weinbroum & Ben-Abraham 2003) .

Table 3. Hyperthermic syndromes, their causative agents and mechanisms of heat production (Reproduced from Halloran and Bernard 2004)

Hyperthermic syndrome	Causative drugs	Mechanisms of heat production
Malignant hyperthermia	**Anesthetic gases**: Halothane, effeurage, isoflurane, methoxyflurane, cyclopropane, diethyl ether, ethylene. **Muscle relaxant:** succinylcholine, decamethonium, gallamine.	Dysfunction calcium channels of skeletal muscle permit uncontrolled release of Ca^{+2} into the cells. This results in high production of ATP in the mitochondria, leading to muscle contraction and increased metabolism and consequently increased heat production.
Anticholenergic poisoning	Antihistamines, atropine, belladonna alkaloida, benztropine, carbamazepine, chlorpheniramine, clozapine, diphenyhdramine, doxylamine, disopyramide, glutethimide, glycopyrrolate, hydroxyzine, hyoscyamine, meclizine orphenadrine, phenothiazines, procainamide, quinidine, quinine, tricyclic antidepressants.	Hyperthermia is due to central and peripheral muscarinic receptors blockade. Central blockade is due to drugs permeable to the BBB and leads to seizers and coma. Peripherally, it interferes with cutaneous heat loss by impairing sweat gland function.
Serotonin syndrome	L-tryptophan, amphetamines, cocaine, Ecstasy, fenfluramine, mescaline, psilocin, L-dopa/carbidopa, lithium, lysergic acid diethylamine, selective serotonin uptake inhibitors, tricyclic antidepressants, meperidine, dexamethorphan, monamine oxidase inhibitors.	It alters the levels of serotonin and dopamine in the CNS. Heat production is due to agitation, tremor and muscle rigidity.
Neuroleptic malignant syndrome	**Neuroleptics**: phenothiazines, butyrophenones, thioxanthenes. **Tricyclic antidepressants** **Monoamine oxidase inhibitors** **Benzodiazepines**	Drugs alter the central hypothalamic regulation by elevated serotonin and decreased dopamine, causing muscle rigidity and increased heat production.
Sympathomimetics syndrome	Cocaine, ecstasy, amphetamines, methamphetamines.	Heat production involves central and peripheral disturbances in thermoregulation. They impair cutaneous heat loss through vasoconstriction, increased muscle activity and rigidity. It can increase non-shivering thermogenesis through increased hepatic metabolism.

4.1.1. Malignant hyperthermia

One of the well-described syndromes of drug-induced fever is malignant hyperthermia (MH). MH is an autosomal dominant pharmacogenetic clinical disorder characterised by hypermetabolic state and skeletal muscle rigidity after exposure to inhalational anaesthetic gases and succinylcholine (Ording 1989). It was first described by Denborough and his colleagues who found an autosomal dominant pattern in deaths of patient's relatives upon exposure to anaesthetics agents (Denborough et al., 1962).

4.1.2. Pathophysiology of malignant hyperthermia

This hypermetabolic syndrome is caused by an abnormal, sudden and progressive release of calcium ions from the sacroplasmic reticulum to the sarcoplasm (myoplasm). The release of calcium ions in the skeletal muscle leads to actin and myocin interaction which results in muscle contraction. The release of the calcium takes place via the calcium release channel. Muscle relaxation occurs when the calcium returns to the sacroplasmic reticulum through the membrane adenosine triphosphatase (ATPase) pump (McCarthy 2004).

During malignant hyperthermia episodes, calcium transport is mediated by the ryanoidine receptor, isoform 1(RYR1), a Ca^{+2} release channel. It is located in the membrane of the sarcoplasmic reticulum and is directly affected by depolarisation via the transverse tubule system which causes change in the dihydropyridine calcium channel (DHP), the voltage sensor of the skeletal Ca^{+2} release channel. A defect in the ryanoidine receptor allows abnormal calcium release when exposed to anaesthetic gases. The mechanism by which anaesthetic gases cause and initiate abnormal calcium release is unknown (Figure 10) (Litman & Rosenberg 2005).

Uncontrolled calcium release induces skeletal muscle contraction, activates glycogenolysis and oxidative phosphorylation cycle and increases cell metabolism. Sustained hypermetabolism results in heat production, excessive lactate production, high oxygen consumption and increased CO_2 production followed by ATP depletion. This presents as acidosis, hypercapnia and hypoxia (Eyer & Zilker 2007; Jurkat-Rott, McCarthy & Lehmann-Horn 2000). If untreated, continuous myocyte death and

rhabdomyolysis results in leakage of calcium, potassium, creatinine phosphokinase and myoglobin into the circulation. This result in arrythemias, disseminated intravascular coagulation, pulmonary and cerebral oedema, end-organ failure and death (McCarthy 2004; Rosenberg et al., 2007).

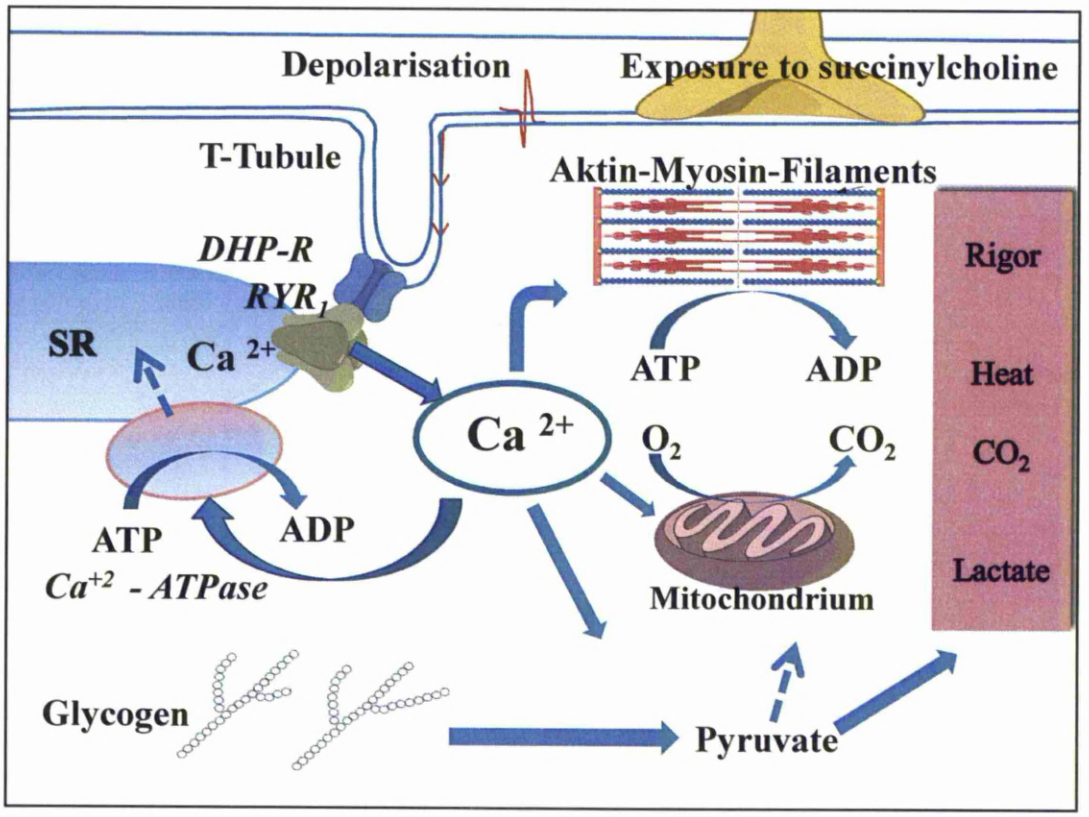

Figure 10. Mechanisms and consequences of uncontrolled calcium release in the skeletal muscles. L-type calcium channel also called dihydropyridine receptor (DHP-R), transverse tubule (T-Tubule), (RYR_1) the calcium release channel also called ryanodine receptor, sarcoplasmic reticulum (SR). Reproduced from (Litman & Rosenberg 2005)

4.1.3. Genetic background of MH

Over 100 mutations have been identified for the RYR-1 gene which lies on the chromosome 19q13.1. However, this gene is not always abnormal in families who are affected by MH. RYR-1 protein (the calcium release channel protein) has a tetrameric structure which bridges the gap between sacroplasmic reticulum and the t-tubulin in skeletal muscle and acts as a calcium release channel. The human RYR-1 gene contains 106 exons, and the 5038 amino acid proteins in the N-terminal domain form a large foot region that contains most of the ligand binding sites. A fifth of the molecules in the C-terminal form the trans-membrane calcium channel (McCarthy, Quane & Lynch 2000).

The majority of RYR-1 mutations are clustered within three regions: the amino acid residues 35 to 614 in the N-terminus, amino acids 2163 to 2456 within the sacroplasmic reticulum membrane and amino acids 4214 to 4806 in the C-terminus (Jurkat-Rott, McCarthy & Lehmann-Horn 2000). Screening of the entire coding region of the RYR-1 genes showed 31 mutations; 16 of them were novel and some of them were located outside the main three regions. All the 31 mutations were localised in 23 exons out of the 106 exons of RYR1 gene (Galli et al., 2006).

In addition to RYR-1 gene mutation, at least other five mutations have been discovered in association with MH, but only one gene has been identified (Table 4). The CANCA1S or CACNL1A3 gene, that encoding for the alpha subunit of the DHP channel (DHPR), is the second gene discovered with mutation associated with MH (Rosenberg et al., 2007). The mutations and chromosomal locations are shown in Table 6 (Greenbaum, Weigl & Pras 2007).

Table 4. Loci, chromosomal location and genes involved in malignant hyperthermia (Greenbaum, Weigl & Pras 2007)

Locus	Chromosomal location	Gene
MHS1	19q13.1	RYR1
MHS2	17q11.3-q24	Unidentified
MHS3	7q21-q22	Unidentified
MHS4	3q13.1	Unidentified
MHS5	1q32	CANCA1S
MHS6	5p	Unidentified

4.2. Molecular aspects of hyperpyrexia (fever)

The pyrogenic effect of a pathogen or a chemical product may occur through several mechanisms and involves many immunological and physiological products. Cytokines and prostaglandins both contribute to the mechanism of fever. This section shows the role of cytokines and prostaglandins in the development of fever.

4.2.1. Role of cytokines in the mechanism of fever

Cytokines are members of the class of immunopolypeptides produced mainly by mononuclear phagocytes as a result of their activation by invading pathogens and/or their products. These molecules can be synthesised and mediate their action peripheral in the immune system and they can also be released in the circulation and transported to mediate their effect in the central nervous system.

Several cytokines have been investigated for their pyrogenic effect. Interleukin 1 beta (IL1-β), interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α) are considered to be endogenous pyrogens (Conti et al., 2004). A large amount of evidence supports the role of inflammatory cytokines in the manifestation of the febrile response. These cytokines appear in the circulation in response to injection of lipopolysacharides (LPS) at the same time the fever develops. TNF-α is the first cytokine that appears in the circulation (Jansky et al., 1995), followed by traces of IL-1 (Jansky et al., 1995) and a significant amount of IL-6 (Roth et al., 1993). Levels of IL-6 show the best correlation with changes in the body temperature.

Systemic injection of individual cytokines can induce fever. This was proved for IL-1 (Anforth et al., 1998), IL-6 (Blatteis et al., 1990) and TNF-α (Goldbach et al., 1996). Neutralising or antagonising the biological activity of cytokines, such as treatment with IL-1 receptor antagonist (Luheshi et al., 1996), a TNF-α neutralising receptor (Roth et al., 1998) or IL-6 neutralising IL-6 antibodies (Cartmell et al., 2000), resulted in attenuation of febrile response to LPS. Furthermore, experiments in cytokines deficient knockout (KO) mice (Leon 2002), particularly IL-1, showed that LPS-induced fever is reduced but not abolished completely (Kozak et al., 1995). This means that IL-1 contributes to but is not essential for the febrile response to LPS. Moderate LPS-induced fever was completely eliminated in IL-6 KO mice (Kozak et

al., 1998). This suggested that the expression of IL-6 receptor is essential for the response to fever (Chai et al., 1996). This large body of evidence supports the view that appearance of exogenous pathogen in a host causes fever via formation of endogenous pyrogens (cytokines) which mediate their action in the brain.

Cytokines are large hydrophilic peptides with a molecular weight of 15–25 kd. Therefore, they cannot pass the relatively impermeable blood–brain barrier to stimulate the hypothalamic thermoregulatory structures. Three mechanisms have been proposed for the mechanism of immune-to- brain signalling through circulating cytokines. First, cytokines may reach their site of action through gaps in the tight blood-brain barrier which are known are as the circumventricular organs (CVOs). Alternatively, they may interact with their receptors on the endothelial cells or perivascular cells or may pass the blood-brain barrier by active transport system (Roth et al., 2006).

CVOs are so named because they are positioned at distinct sites around the margin of the ventricular system of the brain and they have dense vascularisation, lack a blood-brain barrier and are in contact with the cerebroventricular system. CVOs have a sensory subgroup which includes the organum vasculosum of the laminae terminalis (OVLT), the subfornical organ (SFO) and the area postrema (AP). The sensory CVOs are directly exposed to the circulating molecules which they may be able to sense via specific receptors (Roth et al., 2004). The OVLT is a very important structure because of its location in close proximity to the preoptic area in the anterior hypothalamus which is considered as a major pyrogenic zone of the brain. The role of the OVLT in the mechanism of fever has been investigated in studies on brain lesions. Large lesions that included the OVLT prevented the development of fever after systemic injections of LPS (Blatteis et al., 1983). There is evidence that the OVLT acts as a sensor for circulating endogenous pyrogens. This evidence includes the presence of IL-1 (Ericsson et al., 1995), IL-6 (Vallieres & Rivest 1997) and TNF-α (Nadeau & Rivest 1999) receptors in the OVLT cells. Also, neurons in the OVLT change their firing rate in response to the cytokines (Shibata & Blatteis 1991) (Figure 11).

Furthermore, the entire brain endothelium is a target for the circulating cytokines. Endothelial brain cells express IL-1 and TNF-α receptors. IL-1 receptor type 1 is predominantly expressed in the venules but not in the arterioles (Konsman et al., 2004), and IL-6 glycoprotein receptor is expressed in the endothelial brain cells under inflammatory conditions (Vallieres & Rivest 1997). The final activation of the brain endothelial and perivascular cells results in the appearance of enzymes that are responsible for the formation of prostaglandin E_2, which is regarded as the principal mediator for fever (Cao et al., 2001).

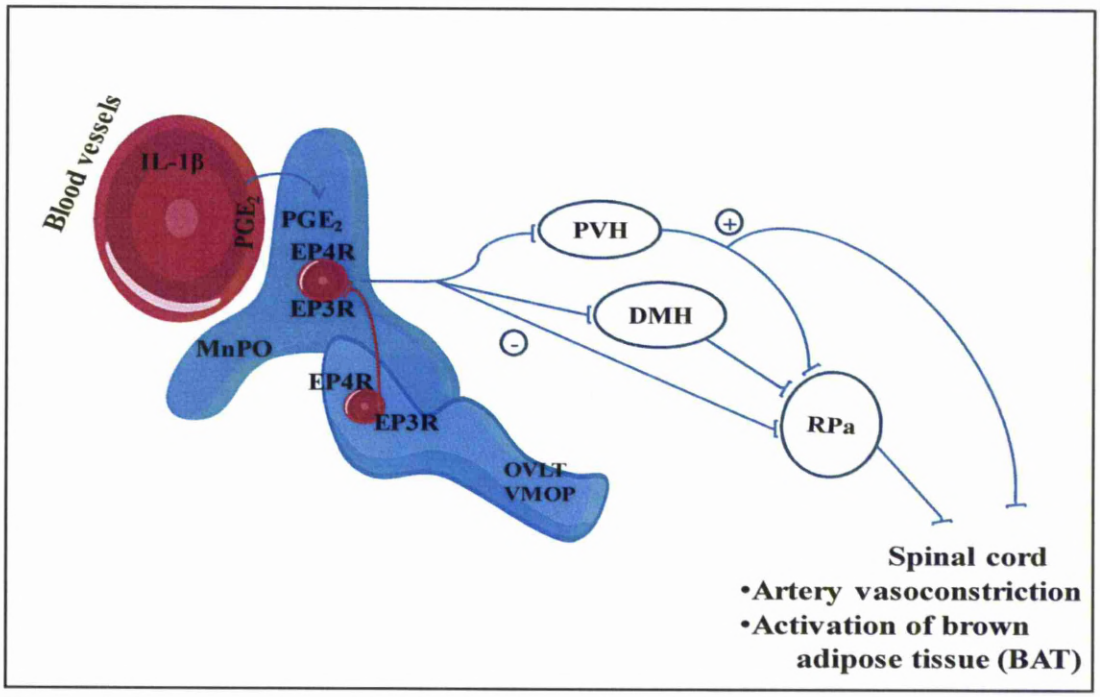

Figure 11. Model shows the role of the preoptic area (MnPO, OVLT/VMPO) in the hyperthermic response. (MnPO – median preoptic area; OVLT – organum vasculosum of the lamina terminalis; VMPO – ventromedical preoptic nucleus; DMH – dorsomedial hypothalamus; RPa – raphe pallidus nucleus). Reproduced from Lazarus (2006)

4.2.2. Role of prostaglandin E_2 in the mechanism of fever

Prostaglandin E_2 is a derivative of arachidonic acid which is produced from diacylglycerol and phospholipid by phospholipase C or phospholipase A2. Arachidonic acid is converted to prostaglandin H2 (PGH_2) by PGH_2 synthase (COX-1 or 2 and peroxidise). PGH_2 is isomerised to PGE_2 by prostaglandin synthetase (PGES) (Ivanov & Romanovsky 2004).

PGE_2 is considered as the key fever mediator in the brain and as the agent that is ultimately in charge of the upward shift of the thermoregulatory set-point. This information was supported by some evidence. Firstly, injection of prostaglandin into the PGE_2 sensitive site within the preoptic nucleus in the hypothalamus stimulates the febrile response (Scammell et al., 1996). Secondly, PGE_2 levels increase in the brain and in the blood in accordance with the changes in the body temperature. Thirdly, systemic administration of drugs that block prostaglandin synthesis pathway inhibits fever effectively. The latter was confirmed by systemic treatment with prostaglandin synthesis inhibitors such as cycloxygenase (COX) inhibitor (diclofenac) or COX-2 inhibitor (meloxicam) which resulted in effective attenuation of LPS-induced fever in rats (Ivanov & Romanovsky 2004).

Many enzymes responsible for PGE_2 formations are induced and up-regulated in response to exogenous and endogenous pyrogens. COX-2 and PGES-1 are inducible enzymes that are regulated by a transcription factor (NK-κB), which is activated by LPS, IL-1 or TNF-α.(Turrin & Rivest 2004). The expression of these enzymes is up-regulated by pyrogenic cytokines in LPS-induced fever to increase production of PGE_2 in the brain and their expression increases predominantly by endothelial brain cells and perivascular cells. Systemic administration of LPS causes strong expression of COX-2 in the OVLT and SFO (Roth et al., 2006). Studies of KO mice deficient in either COX-2 or mPGES-1 showed that febrile response is depressed and therefore, the synthesis of these enzymes is critical for formation of PGE_2 during fever.

PGE_2 mediates its effect via prostaglandin receptor subtype EP3. Studies in KO mice suggested that response to LPS-induced fever is strongly impaired in mice deficient in this receptor. PGE_2 activates EP3 receptors and stimulates the thermogenesis via

activation of the descending neuronal pathway that affects the thermoregulatory set-point in the hypothalamus (Ushikubi et al., 1998).

5. Molecular aspects of prostaglandin-induced hyperpyrexia (fever)

Prostaglandins can be part of the mechanism of hyperpyrexia due to other pathogens and also they can produce fever as a result of their direct effect on the thermoregulatory set-point in the hypothalamus. Therefore, some prostaglandin drugs can cause fever as an unwanted drug reaction. For instance, shivering and fever have been reported in several studies investigating the pharmacological effects of misoprostol in different obstetric and gynaecological indications (Bugalho et al., 2001; Zuberi et al., 2008). The effect of prostaglandin drugs on the human physiological and immunological processes takes place through the prostaglandins pathway, including prostaglandin transport, effect on the cells (target) and the drug metabolism.

5.1. Prostaglandin receptors involved in prostaglandin-induced fever

5.1.1. Prostaglandin transporter (PGT)

Rapid penetration of PGs cannot be achieved by rapid diffusion alone because cell membrane is not permeable to prostaglandins (Bito & Baroody 1975) and there is evidence for the need and presence of carrier-mediated PG transport across the plasma membrane. PGT is the first discovered and the best characterised transporter. It is widely expressed in various peripheral tissues and in the brain of several mammalian species and humans (Schuster 2002). It is localised on the cell membrane and facilitates PG transport in the apical to basolateral direction. The two most specific trans-membrane carriers of PGE_2 are PG transporter (PGT, OATP) (Kanai et al., 1995) and multi-specific organic anion transporters (MOAT, ABCC4) (Nishio et al., 2000).

5.1.2. Prostaglandin receptors (EP)

The four EP receptor subtypes differ in their structure, ligand-binding affinity and signal transduction pathway. PG binding sites are mainly in the plasma membrane and sacroplasmic reticulum (Hamon et al., 1993). EP1 receptors are responsible for contraction of smooth muscle in several tissues including the respiratory tract, the gastrointestinal tract, vas deferens, iris smooth muscles and myometrium. EP3 receptors are involved in mediating contraction of the myometrium, inhibition of the gastric secretion, lipolysis in adipose tissue, modulation of neurotransmitter release and sodium and water reabsorption in kidney tubules (Negishi, Sugimoto & Ichikawa 1995).

EP2 and EP4 receptors are widely distributed in smooth muscle and mediate relaxation of trachea and ileum circular muscles and vasodilatation of blood vessels. These receptors are highly expressed in ileum followed by thymus, lung, spleen, heart and uterus (Lebel et al., 2004). However, the EP2 receptor is not as widely distributed as the other EP receptors (Sugimoto & Narumiya 2007). EP3 receptor mRNA is highly expressed in the kidney and uterus and also expressed in stomach, thymus, spleen, lung and brain. It is also the most abundant EP subtype in the brain and neurons (Sugimoto et al., 1994). EP2 and EP4 receptors are the only two types of PGE_2 receptors expressed by human monocytes. EP2 and EP4 receptors mediate their action via coupling to G proteins of the Gs subclass which increases the intracellular level of cyclic adenosine monophosphate (cAMP) (Panzer & Uguccioni 2004).

EP1 and EP3 receptors are classified as excitatory, whereas EP2 and EP4 are thought to be inhibitory. The inhibitory effect of EP2 is mediated through the activation of adenylate cyclase and increased levels of intracellular cAMP, while EP4 receptors stimulate adenylate cyclase via G proteins (Negishi, Sugimoto & Ichikawa 1995). The stimulatory effect of EP1 and EP3 receptors involves phospholipase C (PLC) activation and mobilisation of calcium from intracellular stores via enhancing inositol 1,4,5-trisphosphate (InsP3) production and also by stimulating calcium influx through the cell membrane (Coleman, Smith & Narumiya 1994). In addition, EP3 receptors mediate its myometrial stimulatory effect by inhibiting cAMP production. Uterine

contraction takes place as a result of increasing intracellular calcium level, which leads to myosin light chain kinase activation. On the other hand, relaxation of uterine muscles is promoted by cyclic nucleotides (cAMP and cGMP), which decreases the affinity of myosin light kinase for calcium-calmodulin complex and by stimulating a mechanism to decrease intracellular calcium levels (Asboth et al., 1996). EP3 receptors are the most widely expressed in the uterine body. The expression of EP3 receptor mRNA is reduced in early pregnancy compared to the non-pregnant uterus. The effect of misoprostol at term may also involve different PGE_2 receptors, particularly EP2 which is more abundant in the lower uterine segment than EP3 receptors (Lyons et al., 2003). EP3 receptors are further subdivided into rEP3α and rEP3β. The receptor rEP3α causes myometrial contraction by inhibiting cAMP formation whereas rEP3β receptors act through calcium mobilisation. High doses of misoprostol produce rapid expression of rEP3α and rEP3β receptor mRNA in the myometrium, but do not change the level of their expression in the cervix, which appears to be low (Lyons et al., 2003).

In the nervous system, the EP3 receptor is highly expressed in the dorsal root ganglion and brain regions such as preoptic area (POA), hypothalamus, hippocampus, maxillary body, locus coeruleus and raphe nuclei (Coleman, Smith & Narumiya 1994). Oka and his colleagues mapped the EP receptors distribution in the brain by *in situ* hybridisation for their mRNA. They found that EP3 is mainly localised to the MnPO (median preoptic nucleus) which appears at the dorsal levels of an inverted Y-shaped structure capping the OVLT (Oka et al., 2000). Apart from EP3 receptors, EP4 receptors are expressed in the VMPO (ventromedical preoptic nucleus) and the lateral OVLT and also located in the MnPO (Figure 11) (Lazarus 2006).

Gamma-aminobutyric acid receptor (GABA receptors) and drenergic, beta, receptor (ADRB1, 2, 3) will be discussed later with the mechanism of prostaglandin induced fever. Table 5 shows summary of the receptors that may involve in the prostaglandin pathway.

Table 5. Summary of different receptors that involved in the prostaglandins pathway, including prostaglandin transport and function

Prostaglandin transporter receptors	Prostaglandin target receptors
Organic anion transporter family, member (influx transporter) (OATP2A1, OATP1B1)	Prostaglandin E receptor (PTGER1,2,3,4)
ATP-binding cassette, sub-family C, member 4 (efflux transporter) (ABCC4)	Gamma-aminobutyric acid receptor (GABA)A
	Adrenergic, beta, receptor (ADRB1,2,3)
	Adrenergic, beta, receptor kinase 1 (ADRBK1)

5.1.3. Misoprostol and prostaglandins receptors

Misoprostol binds to PGE_1 and PGE_2 receptor through its four stereoisomers. Only one isomer is responsible for the receptor-mediated activity, whereas the other three isomers are inactive. Misoprostol is more stable in vivo and has a longer elimination half-life than naturally occurring prostaglandins (Shield 1995). Prostanoid receptors have been classified further to DP, EP, FP, IP and TP (Kennedy et al., 1982). The variable effects of prostaglandins (PG) are explained by the presence of G-protein coupled receptors which are classified into four subtypes of EP receptors (PGE_2): EP1, EP2, EP3 and EP4. Misoprostol is structurally related to PGE_1. It has clear EP2 agonist activity, weak EP1 receptor affinity, but potent and selective EP3 activity (Coleman, Smith & Narumiya 1994). Prostanoid sub-family and its relation with misoprostol is shown in Figure 12.

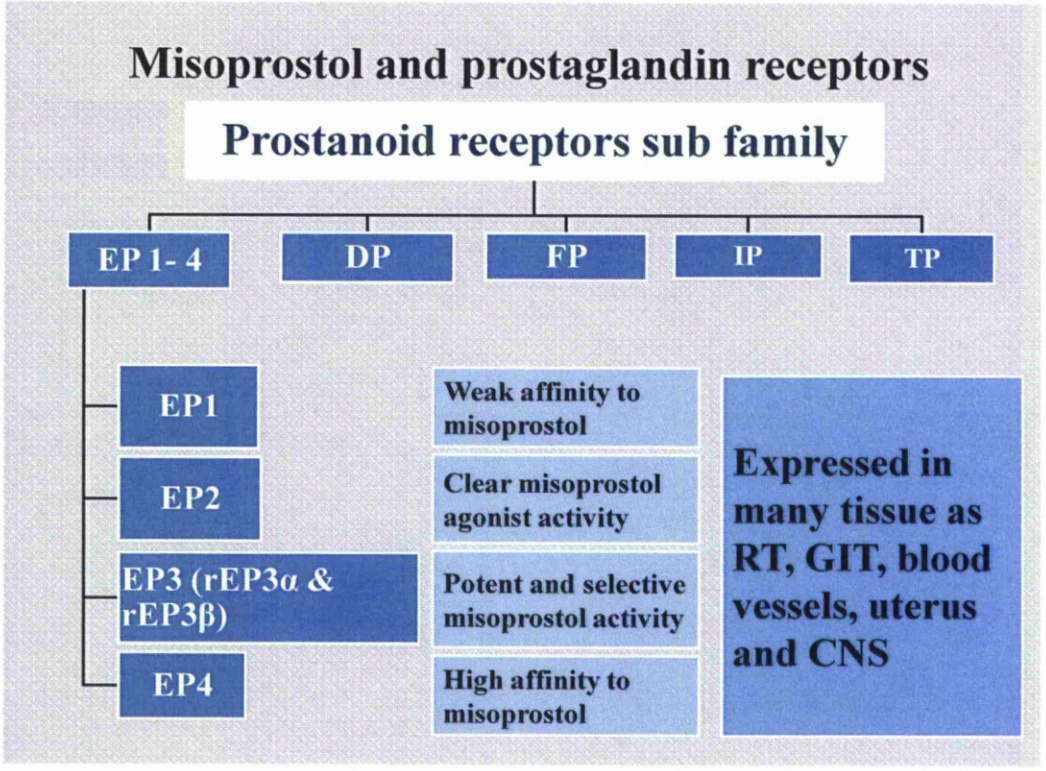

Figure 12. Misoprostol and prostaglandins receptors target (RT – respiratory tract), (GIT – gastrointestinal tract), (CNS – central nervous system)

6. Mechanisms of prostaglandin-induced fever

PGE2 is considered as a principal mediator of fever in the brain and a key intermediate in the sequence of events leading to fever. Therefore, it is essential to understand the mechanism of fever in general and the important role of prostaglandins as a mediator of fever due to other pyrogens and also as substance inducing fever.

The thermogenic activity of prostaglandin E has been recognised in humans after increasing its application in reproductive medicine. The administration of PGE and its analogues by different routes and at variable doses provoked thermal side effects. It was reported early (1978) that the incidence of elevated body temperature was 53%, shivering was 93% and subjective coldness was 100% (Dingfelder & Brenner 1978). A recent randomised controlled trial has confirmed that shivering and pyrexia are the most common side effects of 600 mcg oral misoprostol. The incidence of shivering and pyrexia were 44% and 38% respectively (Hofmeyr et al., 2001b).

Prostaglandin (E-type) has been assumed as a principal mediator of fever in the brain (Boulant 2000; Kanosue, Yanase-Fujiwara & Hosono 1994; Kanosue et al., 1994) and it circulates as albumin-bound. Once it is dissociated from albumin at the blood-brain barrier (BBB), it is carried into the brain by prostaglandin transporters (PGTs) and organic anion transporting polypeptide subtype 2 (Oatp2) expressed at the BBB (Taogoshi et al., 2005). Many studies have been conducted to investigate the role of prostaglandin E1 in the production of fever in cats and rabbits (Feldberg & Gupta 1973; Feldberg & Saxena 1971; Stitt 1973). Microinjection of PGE_1 into the anterior hypothalamus of unanaesthetised cats raised the core body temperature. On the other hand, even the injection of a larger amount of PGE_1 into the posterior hypothalamus did not affect the temperature (Feldberg & Saxena 1971). The same conclusion has been drawn by Stitt (1973) when the same experiment was conducted on rabbits. The site where PGE1 acts to produce fever is the same as the site where pyrogens produce their thermogenic effect (Stitt 1985). This would support the idea that PGE_1 is a mediator of pyrogen-induced fever. Furthermore, PGE_1 febrile effect is not an all or none response. It is in a dose-responsive manner, like in pyrogenic fever. Both pyrogen and PGE_1 cause an elevation of the thermoregulatory set-point in the hypothalamus (thermostat) resulting in cold feeling, increased heart rate, muscle tone

and shivering. The change in the temperature due to prostaglandin and pyrogen is a regulated one. While pyrogen is regarded as a precursor of PGE_1 in the brain tissue, PGE_1 is one step closer to the production of fever. Therefore, the onset of PGE_1 fever is more rapid than pyrogen (Stitt 1973).

PGE_2 is a powerful mediator of pyrogen-induced fever in the brain and mainly in the POA. The evidence showed increased levels of PGE_2 in the cerebrospinal fluid after pyrogen-induced fever (Sirko, Bishai & Coceani 1989). The injection of PGE_2 into (OVLT) and (POA) induce elevation of the core body temperature and its effect is mainly mediated through EP1 and EP3 receptors (Oka 2004).

EP2, EP2/EP3 agonists produce a gradual rise in body temperature, whereas the effect of EP1 agonist was not significant. EP3 agonist showed marked febrile response, suggesting the significant role of EP3 receptor in mediating febrile response. This response has been reported after injection of prostanoid receptor (EP1, EP2, EP3 subtypes) agonists into the lateral ventricles of prepubertal pigs which produced dose-related increase in body temperature (Parrott & Vellucci 1996). In addition, a study of EP receptor knockout mice showed lack of a febrile response to PGE_2 in EP3 receptors gene-deleted mice which indicate the crucial role of EP3 receptors in producing fever (Lazarus et al., 2007; Ushikubi et al., 1998). Also, EP3 receptors KO mice showed a profound hypothermia, with decrease in body temperature up to 6°C (Oka et al., 2003). This shows that EP3 receptors are important to prevent a drop in temperature which occurs as a result of EP4 receptors' response to PGS. On the other hand, PE1 receptor-deficient mice showed mild and partial suppression of the febrile response. This indicates that all EP receptors are involved in the thermoregulatory effect of PGE_2 and each receptor may have a different role (Oka, Oka & Saper 2003).

The preoptic area (POA) is considered as the sole region that can sense PGE series-induced fever. PGEs produce their thermogenic effects mainly through PE3 receptor-containing neurons in the somatodendritic portion of POA, specifically in the MnPO and MPO subregions. These neurons have direct input to DMH, LH, DH and RPa and so mediated their sympathetic thermogenesis (Nakamura et al., 2002). However, little information is available about the molecular events of PE3 activation in the preoptic neuron. Many studies suggested that GABA and $GABA_A$ receptors are essential for

prostaglandin-induced febrile response in the POA. Microinjection of a $GABA_A$ receptor antagonist, bicuculline and gabazine into the POA area unilaterally, either contraleteral or epsilateral to the site of PGE_2 injections, results in complete blockade of the PGE_2-induced thermogenesis (Osaka 2008a). This finding indicates that the tonic GABAergic transmission to POA neurons has a role in the full expression of PGE_2-induced fever. In normal situations (in the absence of PGE_2), EP3-expressing neurons produce a strong GABAergic tonic inhibition on fever-mediated raphe pallidus neurons (rRPa) and DMH neurons. PGE_2 suppresses the GABAergic activity of EP3-expressing neurons, resulting in disinhibition and activation of the rRPa and DMH neurons, leading to thermogenesis (Nakamura et al., 2005). The above mentioned mechanism of prostaglandin-induced fever is summarised and simplified in Figure (13).

PGE_2-EP3 signalling may produce the GABA inhibition through changing the $GABA_A$ channels properties. It may affect the sensitivity of these channels to GABA or the response of the POA neurons to GABA. EP3 receptor binds to $G_{i/o}$ proteins in the POA neurons and results in attenuation of *gabr* gene expression. It has been demonstrated that PGE_2-EP3 signalling rapidly decreases the expression of $GABA_A$ gene. As a result of the reduced number of $GABA_A$ receptors, the EP3-expressing neurons could become insensitive to the inhibition from GABA and mediated PGE_2-induced fever (Tsuchiya et al., 2008).

On the contrary, other studies have defined a region adjacent to the OVLT (peri-OVLT) in the ventromedial POA as the main site for the PGE-induced febrile response (Ranels & Griffin 2003; Stitt 1991). The same GABAergic effect in the DHM and RPa has been proved to take place in the peri-OVLT region (Osaka 2008b). Variation in these studies' findings can be attributed to the different microinjection technique, chemicals used for injection and more to the interrelation of neurons in these very adjacent areas in the hypothalamus.

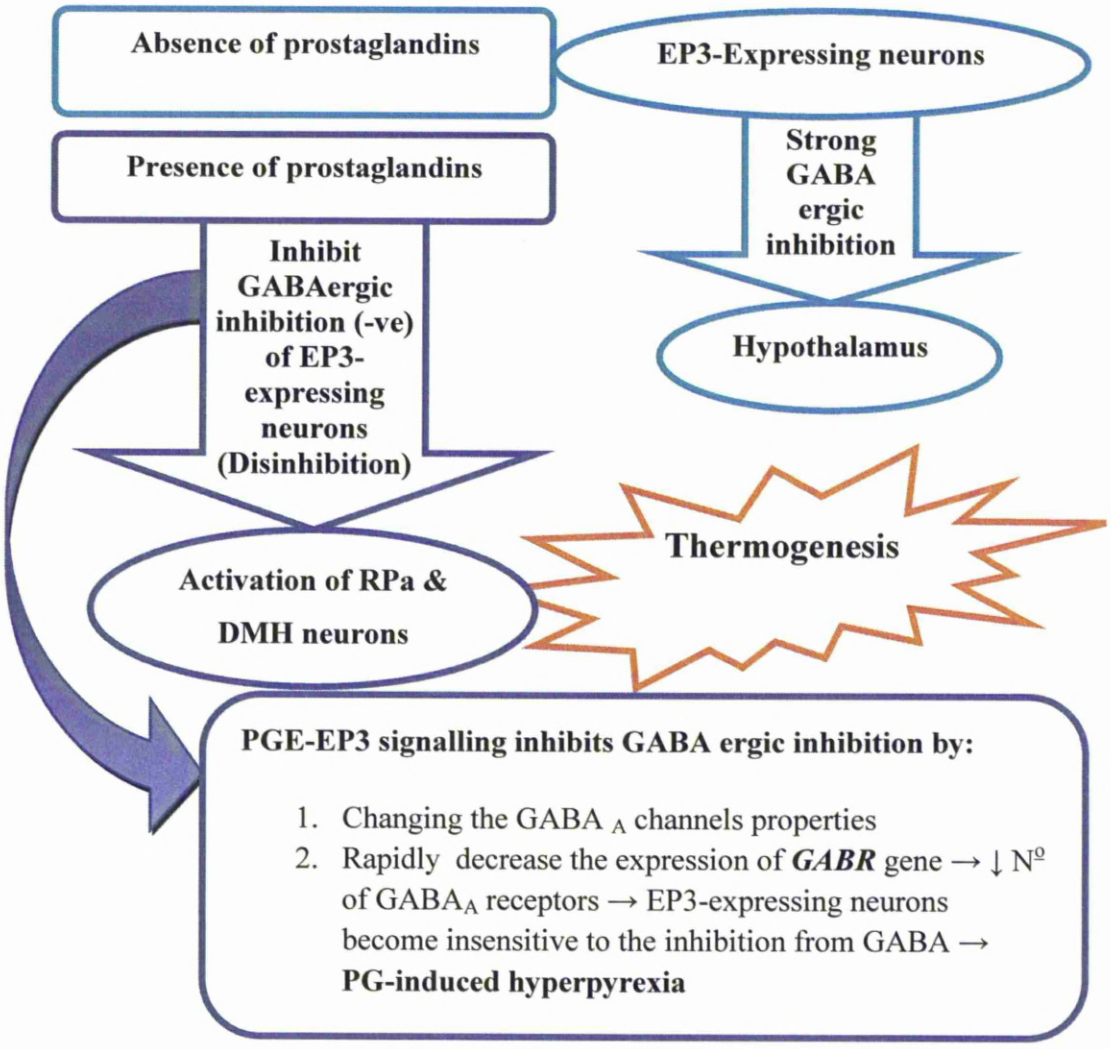

Figure 13. Summary of the mechanism of prostaglandin-induced fever as described in Nakamura et al. 2005

Prostaglandins hyperpyrexia can also be produced by non-shivering thermogenesis via stimulating brown adipose tissue (BAT). The significant role of BAT in PGE_1-induced fever is confirmed in rats (Fyda, Cooper & Veale 1991). BAT, which is mainly localised in the interscapular region, is under control of the peripheral sympathetic nervous system (Foster, Depocas & Zaror-Behrens 1982; Foster, Depocas & Zuker 1982). PGS acts through the central nervous system to modify the sympathetic outflow to BAT. This is confirmed after intracerebroventricular (ICV) injection of PGE_1, which results in increasing the sympathetic tone in the nerves innervating the BAT (Monda, Amaro & De Luca 1994). PGE_2 is also involved in the non-shivering thermogenesis mechanism (Figure 19) and is considered as a potent stimulant for BAT thermogenesis. The injection of PGE_2 into the anterior hypothalamic preoptic area (POAH) can produce a rapid and sustained increase in BAT and core temperature via increasing BAT thermogenesis through sympathetic drive from the VMH to BAT (Amir & Schiavetto 1990). BAT heat production is under sympathetic nervous system control through sympathetic norepinephrine stimulation of B-adrenergic receptors on the brown adipocytes membrane. Inhibition of the B-adrenegic receptor by propranolol or pre-treatment with sympathetic ganglionic blocker chlorisondamine chloride completely prevented the increase in the BAT and core temperature induced by PGE_2 injection to the POAH (Amir & Schiavetto 1990). The thermoregulatory signals from intra-POAH injections of PGE_2 can be mimicked by the signals produced by cold stimuli or cooling of intra-POAH (Imai-Matsumura & Nakayama 1987). These signals involve inhibition of warm-sensitive neurons and stimulation of cold-sensitive neurons in the POAH, resulting in increasing the thermoregulatory set-point in the hypothalamus (Satinoff 1978).

7. Pharmacogenetics and adverse drug reactions

Individuals' variations in response to medications may be caused, at least in part, by differences in their genetic profile. Pharmacogenetics and pharmacogenomics are often used interchangeably to describe the genetic approach to explain the individual's drug response. According to The European Agency for the Evaluation of Medical Products' position paper on terminology (EMEA 2002), the term pharmacogenetics is defined as the study of inter-individual variations in DNA sequence related to drug

response, whereas pharmacogenomics is used to describe the study of the variability of the expression of individual genes relevant to disease susceptibility as well as drug response at cellular, individual or population level. Pharmacogenomic research strategies involve isolation and evaluation of the genetic polymorphisms in individuals and the establishment of definitive relationships between these genetic variations and drug responses (McLeod & Evans 2001).

The most common sequence variations in the human genome are single nucleotide polymorphisms (SNPs, pronounced *snips*), where one base pair in the DNA sequence is replaced with another (e.g. C to T). It has been estimated that there are approximately 10 million SNPs in the human genome, but only approximately 60,000 are located in the coding regions of genes, and only half of these affect the amino acid sequence of the protein produced (Risch 2000; Sachidanandam et al., 2001). However, SNPs do not have to be located in the coding sequence to have an effect on disease. SNPs in promoter (Klerkx et al., 2003; Lin et al., 2003), intron (Tokuhiro et al., 2003), splice site (Betticher et al., 1995) and intragenetic regions (Helms et al., 2003) have been associated with disease or susceptibility to disease (Crawford & Nickerson 2005). A SNP can be isolated individually or identified by looking at groups of genes that are known to be inherited together (haplotype). The width of research can be varied from the entire genome to a candidate gene approach.

7.1. Genome-wide method

The genome-wide method is a comprehensive but expensive method using SNP arrays (GWA). It is a hypothesis-free method and can reveal unexpected SNPs related to drug function or adverse reactions. It does not rely on the current knowledge of the mechanism of action and drug metabolism (Kooloos et al., 2009).

GWA is normally designed as a case-control study to compare the DNA of the two large groups of participants. One is a group of people affected by the disease or a particular drug adverse reaction and the other is a group of healthy individuals who are not affected. Each individual is genotyped for the majority of the common known SNPs. It may include genotyping several millions of SNPs. For each of these SNPs it is then investigated if the allele frequency is significantly different between the case

and the control groups. In such setups, the odd ratio (OR) of interest is the odds of disease (the probability that the disease is present compared with the probability that it is absent) in exposed versus non-exposed individuals. In the context of GWA studies, OR is the proportion of individuals in the case group having a specific allele to the proportion of individuals in the control group having the same allele. If the allele frequency in the case group is much higher than in the control group, the OR will be greater than 1. The significance of the OR is calculated using P values from chi-squared test. Finding ORs that are significantly different from 1 is the objective of the GWA study because this shows that a SNP is associated with disease (Clarke et al., 2011). In addition to the association calculations, it is important to take into account the presence of several confounding factors which affect the results, such as age, sex and the genetic variations with different geographical populations and ethnical backgrounds. Controlling for the genetic and geographical variations is called 'population stratification'. This will show if the disease or the positive results are due to the underlying structure of the population and not due to a disease-associated locus (Freedman et al., 2004).

GWA studies have several limitations. The common problems are insufficient sample size, control of multiple testing and population stratifications. GWA methods and platforms do not provide a complete coverage of all the chromosomes and chromosomal regions. If the platforms cannot sufficiently capture the variation in these genes, some of the causative variants that influence the phenotype may fail to be identified. Also, the coverage of SNPs is incomplete, particularly those with low minor allele frequency (MAF) (e.g. MAF<0.05). Many pharmacogenomic association studies have relatively small sample sizes compared to the disease-associated studies. To overcome the issue of small sample size, detecting the causative SNP may require coverage of the SNP on the GWA's platform, sequencing of the gene, or imputing to the 1000 Genomes dataset. If the causative gene is poorly interrogated on the genome-wide platform, it is more likely to miss important associations. The search for causative variation may require combining several methods to adequately survey all the genetic variation in pharmacogenes. This includes genome-wide study, candidate gene and imputation into sequence data (e.g. 1000 Genomes) (Gamazon, Skol &

Perera 2012). Also, GWA studies are expensive and may be replaced by complete genome sequencing as their price is rapidly decreasing.

7.2. Candidate gene method

A candidate gene is a gene of known biological action involved in the physiological or pathological process under investigation. Candidate gene studies focus on the genes that are involved in the mechanism of the disease or drugs. Candidate gene study is conducted on population-based samples from affected and unaffected people (a case-control study). There are some problems with candidate gene studies as significant findings in some studies have not been replicated and also the current knowledge is insufficient to rely on them for selection and prediction (Tabor, Risch & Myers 2002).

7.2.1. Selecting candidate genes

Candidate gene selection involves an extensive literature search regarding the mechanism of action of a particular drug function, such as genes encoding for transporters and genes encoding target receptors and genes encoding for receptors that are involved in drug metabolism (Kooloos et al., 2009). Also, the search involves published studies of the phenotype of interest and the examined candidate genes. It is important to consider the reason for selecting a particular gene in these studies before selecting it for genotyping. In addition, linkage studies may provide information about genomic regions, population characteristics and phenotypic definition (Tabor, Risch & Myers 2002).

7.2.2. Prioritising polymorphism (SNP selection)

It is not practical or statistically feasible to genotype and test all SNPs in the genome for association with phenotypes. Therefore, it is important to carefully select a limited number of SNPs to genotype from the considerable number that is often available in a particular candidate gene. Polymorphisms that affect the function of protein expression and synthesis are desirable as they are most likely to affect the risk of phenotype.

Information about the location and type of the sequence variants in a gene can be used to prioritise polymorphisms. It involves selection of SNPs with high probability to detect. It includes SNPs in the genetic regions with functional characteristics such as synonymous or promoter or untranslated regions. SNPs in the non-coding regions like introns could have indirect effect on transcription regulatory proteins. In the NCBI database, specific regions within each gene were investigated. There are four subdivisions, unknown, 3′ and 5′ near gene, introns and exons. The latter also were subdivided into synonymous, non-synonymous, 3′ UTR and 5′ UTR subgroups (Table 6).

SNPs with low minor allele frequency (MAF) have a low chance of being detected and need large sample size to achieve a statistically significant association. SNPs causing amino acid alteration (non-synonymous SNPs) and effects on gene expression have been extensively studied. On the other hand, SNPs located within non-coding regions remain poorly identified. If a SNP has a predicted significant effect, it is favourable to include it in the association studies (Tabor, Risch & Myers 2002).

Table 6. Priorities for single-nucleotide-polymorphism selection reproduced from Tabor, Risch & Myers 2002

Type of variant Predicted	Location	Functional effect	Frequency in genome	relative risk of phenotype
Nonsense	Coding sequence	Premature termination of amino-acid sequence	Very low	Very high
Missense non-synonymous (non-conservative)	Coding sequence	Changes an amino acid in protein to one with different properties	Low	Moderate to very high, depending on location
Missense/non-synonymous (conservative)	Coding sequence	Changes an amino acid in protein to one with similar properties	Low	Low to very high, depending on location
Sense/synonymous	Coding sequence	Does not change the amino acid in the protein — but can alter splicing	Medium	Low to high
Promoter/regulatory region	Promoter, 5′ UTR, 3′ UTR	Does not change the amino acid, but can affect the level, location or timing of gene expression.	Low to medium	Low to high
Intronic	Deep within introns	No known function, but might affect expression or mRNA stability	Medium	Very low

7.2.3. Linkage disequilibrium among SNPs

Another important consideration in selecting SNPs for an association study is whether there is significant linkage disequilibrium in the candidate gene. Linkage disequilibrium is the non-random association of alleles at two or more loci, not necessarily on the same chromosome. In other words, linkage disequilibrium is the occurrence of some combinations of alleles or genetic markers in a population more often or less often than would be expected from a random formation. The degree of LD between alleles at two loci can be described with the correlation coefficient (r^2). An r^2 of 1 indicates full linkage, which means there is no loss of power when using a tag SNP instead of directly genotyping the direct SNP associated with the drug reaction. LD blocks (including tagged SNPs) can be relocated using the metric *D'*, which is closely related to r^2 and gives information about the recombination breakpoints of chromosomes (Kooloos et al., 2009). It is also important to search for tag SNPs and particularly those in linkage disequilibrium.. Tag SNPs usually occur in haploblock or subregions, and SNPs in different haploblocks or from different genes are in LD. To limit the cost and effort of association study, taking tag SNPs in account is very important (Kooloos et al., 2009).

Misoprostol-induced fever is a common and well-known side effect. The mechanism of misoprostol-induced fever has not been investigated solely. However, many studies examined the mechanism of prostaglandin-induced fever in animal models. The pharmacogenetics may provide an explanation of the presence of misoprostol-induced fever in certain populations and not in others. This will be investigated in a candidate gene association study in Chapter 4.

III. Postpartum haemorrhage

1. Introduction

Although PPH is considered to be the most avoidable cause of maternal death, and in spite of the global effort to improve maternal health, developing countries are still far away from the Millennium Development Goal 5 (MDG5). This aimed to reduce the global maternal mortality ratio by three quarters between 1990 and 2015, but only a 5% decline in the maternal mortality ratio was achieved between 1990 and 2005. Global maternal mortality has, however, declined from 409,100 (uncertainty interval 382,900-437,900) in 1990 to 273,500 (256,300-291,700) deaths in 2011 (Lozano et al., 2011). In addition to death, PPH also is an important cause of maternal morbidity, causing hypovolaemic shock, disseminated intravascular coagulation (DIC), hepatic failure, renal failure and adult respiratory distress syndrome (Bonnar 2000).

There are several factors that contribute to the high maternal morbidity and mortality from PPH in resource-poor sittings. Women in developing countries are more likely to have severe anaemia during pregnancy, have other serious diseases like malaria and AIDS, and experience difficulties in accessing the health services due to geographical and social factors. Moreover, even if the women can reach the health facilities still they may face difficulties due to a lack of adequate medical supplies including blood products. For example, in Uganda, 97% of health facilities that expected to be able to offer emergency obstetrics care had shortages in the basic services (Mbonye et al., 2007). A great deal of effort has been put into the identification of women at risk of PPH on the basis of historical and clinical factors. However, more PPH occurs in women without risk factors than in women who have identifiable risk factors. It is recommended, therefore, that all women during child birth should have access to PPH management, as it often occurs without warning (WHO 2007).

This section, will give an overview of PPH and its management, starting with the definition, incidence and prevalence and it will highlight the main aetiologies and risk factors of PPH before it turning to details of strategies for its prevention and management.

1.1. Definition of PPH

Although most clinicians have observed PPH, a globally accepted definition is still elusive. The most common definition of PPH is a blood loss greater than 500 ml in the first 24 hours, with more than 1000 ml classified as severe PPH (SPPH). Nonetheless, these cut-offs may be not clinically significant because healthy pregnant woman can tolerate blood loss up to 1000 ml before showing any symptoms or fall in haemoglobin (Hofmeyr & Mohlala 2001). The majority of pregnant women are young and healthy, with a body physiology well adapted to the anticipation of blood loss. This occurs through blood volume expanding by around 50% and the red blood cell volume increasing by 32% (Pritchard 1965).

The amount of blood loss at delivery usually incorrectly estimated (Stafford et al., 2008). Using standard measures to estimate blood loss can improve the accuracy of estimating blood loss, but this approach is not feasible to be applied worldwide. Furthermore, a recent randomised trial has shown that an accurate assessment of blood loss does not assist with management (Zhang et al., 2010). Therefore, another definition of PPH can be used to overcome any mistaken numerical assessment; this is the amount of excessive bleeding that results in signs and symptoms of hypovolaemia (Rajan & Wing 2010). There is another diagnostic criteria for PPH. It includes a hematocrit drop of greater than 10% or bleeding that necessitates blood transfusion or that results in signs and symptoms of hypovolaemia (Combs, Murphy & Laros 1991b) . The Royal College of Obstetricians and Gynaecologists (RCOG) recommended that a primary PPH with an estimated blood loss of 500-1000 ml and no signs and symptoms of shock should prompt basic measures of monitoring and readiness of resuscitation. This includes close monitoring, intravenous access, full blood count, and group and screen. However, an estimated blood loss of more than 1000 ml (or a smaller loss associated with clinical signs of shock, tachycardia, hypotension, tachypnoea, oliguria or delayed peripheral capillary filling) prompts full measures to resuscitate, monitor and arrest the bleeding (RCOG 2009).

PPH is divided into two types. Early PPH (primary) occurs within the first 24 hours after delivery and late PPH (secondary) occurs after 24 hours postpartum but before 6

weeks. The causes of early and late PPH are naturally different. However, most of maternal deaths due to PPH occur immediately after delivery due to acute blood loss.

1.2. Epidemiology of PPH

Reports of the incidence of PPH vary widely due to the use of different diagnostic criteria. However, it is estimated at between 4 and 6%. A systematic review of databases from 1997 to 2006 has been performed to assess the global incidence of PPH (Carroli et al., 2008). The definitions of PPH is a blood loss > 500 ml and SPPH as > 1000 ml blood loss were used as inclusion criteria for studies investigated PPH. On the whole 224 datasets were included in the final analysis. The finding illustrated an overall prevalence for blood loss of over 500 ml of 6.09% [95% confidence intervals (CI), 6.06-6.11] of all deliveries. The rate was 10.6% when blood loss was measured objectively. For SPPH (loss over 1000 ml), the prevalence was 1.86% (95% CI, 1.82-1.90), but 3.04% (95% CI, 2.90-3.17) when the blood loss was measured objectively (Carroli et al., 2008). Interestingly, this extensive systematic review found that PPH was more common in the rural than urban areas. On the other hand, there was no difference in SPPH rate between these settings. They also found a wide discrepancy between the developing and developed countries with the highest incidence in Africa (10.45%) which the authors attributed to a lack of skilled birth attendance and a scarcity of facilities to provide a satisfactory management during labour.

The PPH rates in individual studies vary greatly. For example, a study of 1620 women from rural India with low risk pregnancies found that the rate of measured PPH of over 500 ml was 9.2% (Geller et al., 2008). In a study where PPH was defined as estimated blood loss > 1000 ml, a need for blood transfusion or presence of sign and symptoms of hemodynamic instability found that the incidence of PPH was 5.2% of women who delivered vaginally (Magann et al., 2005). In a case-control study of 9598 vaginal deliveries, the PPH defined as a haematocrit drop of 10 points or more found that the rate of PPH was 3.9% (Combs, Murphy & Laros 1991b) . In another study of women delivered by caesarean section using the same definition of PPH, PPH occurred in 196 of 3052 women, producing a rate of 6.4% (Combs, Murphy & Laros 1991a) .

The rate of PPH is significantly reduced by the administration of prophylactic oxytocin during the third stage of labour as part of the active management of the third stage of labour (AMTSL). Recently 2 large studies in which blood loss was measured in 40,000 women delivering vaginally with view to randomising those who had blood loss of over 700 ml to receive 800 mcg sublingual misoprostol or 40 IU oxytocin intravenous. In 9348 women who did not receive prophylactic oxytocin during vaginal delivery, 978 (10%) cases were diagnosed with PPH (Winikoff et al., 2010). In contrast, PPH complicated the delivery of 809 (3%) of 31,055 women who received oxytocin as a prophylaxis during labour (Blum et al., 2010). Whilst women in these studies were not randomly allocated to receive oxytocin prophylaxis or not, the data does clearly demonstrate the association of oxytocin prophylaxis with lower PPH rates.

Very few studies have investigated the rate of secondary PPH. A retrospective study of 123 women presented with secondary PPH over a three-year period found that secondary PPH complicated less than 1% of deliveries. This was associated with a history of primary PPH and retained placenta, and resulted in considerable maternal morbidity (Hoveyda & MacKenzie 2001).

1.3. Trends in PPH rates

Even though there is little literature available on studies reporting PPH trends, it does appear to be an overall increase in rate of PPH over time. A retrospective study of over 750,000 women was conducted to examine the effect of changes in risk factors and their relationship to rising in PPH rate over the period from 1994 to 2002 in Australia. It found that the reported rate of PPH increased from 4.7% to 6.1%. This increase was not related to changes in the risk factors of the women over time, but the authors suggest that it might be explained by changes in the management and reporting of PPH (Ford et al., 2007b). Similarly, a population- based study in New South Wales in Australia included more than 52,000 women and found that the rate of PPH increased from 8.3% to 10.7% from 1994 to 2002. This change was not associated with changes in the risk factors. However, there was a 6-fold increase in the use of blood transfusion for the management of PPH over the same period (Cameron et al., 2006).

A retrospective cohort study was conducted in Canada to investigate the trends in PPH between 1991 and 2004. The study found an increase in PPH rates from 4.1% to 5.1%, a change that was associated with a 73% increase in the hysterectomy rate. This increase in PPH was explained by a 34% increase in uterine atony over that period. The cause of the increased rate of atonic PPH remains unclear (Joseph et al., 2007).

The rate of manual removal of placenta in the United Kingdom has considerably increased from a mean of 0.66% in the 1920s to 2.34% in the 1980s and this might have an effect on the rates of PPH. The authors postulated that this increase might occur as a result of increased rates of labour induction, premature birth and pregnancy termination with time (Cheung et al., 2011).

1.4. Recurrence of PPH

Previous history of PPH is considered as a significant risk factor for PPH in subsequent pregnancies. A population-based study was conducted in Australia to investigate the occurrence and recurrence of PPH in 125,295 women from 1994 to 2002 (Ford et al., 2007a) . Among the women studied, 5.8% had a PPH in their first pregnancy and 14.8% of these women had PPH in their second consecutive pregnancy, which represents a 3-fold increased risk of PPH (RR 3.3, 95% CI 3.1-3.5). The rate of PPH in a third consecutive pregnancy in women who had a PPH in their first two consecutive pregnancies was 21.7%. Furthermore, the rate of PPH in the third pregnancy of women who had a PPH in their first pregnancy and second uncomplicated pregnancy was 10.2%. Therefore, women with history of PPH in previous pregnancy are at increased risk of PPH in a subsequent pregnancy.

2. Physiology of the third stage of labour

To understand the pathophysiology and aetiology of PPH, it is important to know the mechanical, physiological and coagulative events of the third stage of labour.

2.1. Mechanical events of the third stage of labour

The biomechanical events which lead to the delivery of placenta and membranes starts in the first stage of labour, where detachment of the membranes begins and slowly progresses upwards from the internal os. During the second stage of labour, when the baby's trunk is delivered, the uterine muscles undergo a very powerful contraction. The uterus is reduced in size and volume as the uterine muscle shortens. This process is known as retraction. The latter may be facilitated by the special arrangement of uterine muscle fibres. The outer longitudinal muscle cells are generally arranged in the long axis of the uterus and the inner circular muscle cells are arranged concentrically around the longitudinal axis (Garfield & Yallampalli 1994). The decrease in uterine size and volume and tight compression of the placenta causes a reduction in the placental site surface area. The compression of the placenta also forces the placental blood back into the sinuses in the decidua basalis. The placenta attempts to force blood against the high resistance in the uterine sinuses produced by the strong myometrial contraction. As result, the sinuses become congested and rupture. The blood that escapes from the ruptured sinuses tears the fine septae of the spongy layer of the decidua basalis. Consequently, the placenta shear off (Khan & El-Refaey 2006).

Real-time ultrasonographic imaging studies during the third stage of labour were performed to investigate the mechanism of placental delivery. One study reported that normal third stage of labour occurs in four phases (Herman et al., 1993). It started with a 'latent phase', characterised by thin placenta site wall and thick placental free wall. This is followed by the 'contraction phase' where the retro-placental myometrium increases in thickness from < 1 cm to > 2 cm. When it achieves its full thickness the placenta completes its separation and detaches – this is the 'detachment phase'. Failure of the retro-placental myometrium contraction may cause dysfunctional labour and retained placenta (Weeks 2003). With a sliding movement,

the placenta comes out during the 'expulsion phase'. Interestingly, neither the latent phase nor the contraction phase was associated with presence of retroplacental haematoma formation.

There are two classical methods of placental delivery which result in different bleeding patterns. In the Schultze method, separation and descend occur in the central part of the placenta followed by the rest. The Matthew Duncan method is characterised by detachment of the placenta edges leading to the entire organ sliding down and out of the uterus sideways. There is however little relevance of this; distinction of the placental separation method appears to be clinically irrelevant and clinicians are incapable of predicting or changing the method of separation (Khan & El-Refaey 2006).

A similar classification of the mechanisms of placental separation and expulsion was reported using continuous real time ultrasound during the third stage of labour. Monophasic separation of the placenta, in which placental detachment occurred simultaneously, was uncommon. The most common type of placental separation was (down-up separation) where the process started at the lower pole. It rarely began from the upper pole (up-down separation). In cases of a fundal placenta, the separation was considered multiphasic but it began from either the posterior or anterior pole with the fundal part separating last (bipolar separation) (Herman et al., 2002) . The up-down separation was more common in women who had previous caesarean section, probably due to impaired lower uterine segment contractility (Mo & Rogers 2008).

Another study using real time B mode ultrasound described placental separation in three types. In Type I, which occurred in 53% of the cases, placental separation from its bed occurred smoothly and slides out immediately, usually one or two contractions after the delivery of the baby. This type was characterised by the least blood loss and the shortest duration of the third stage of labour. Placental separation in Type II started at the marginal site and gradually progress with each recurring contraction. With this type, the bleeding tended to be greater and the time to placental separation tended to be longer. In Type III, separation of the placenta began at the central part with formation of retroplacental clots resulting in an increase in placental size. The blood loss and the duration of the third stage was generally moderate (Goto 1984).

The contraction and retraction of the interlacing myometrial fibres plays a vital role in PPH control. When the placenta is separated from the uterine wall it leaves about 300 square centimetres with may be 100 torn arteries which had been delivering around 500 ml of blood per minute to the placenta (Hytten 1995). This massive trauma requires both a physical compression of the blood vessels by the myometrial contraction, as well as vessel blockage by haemostasis. Uterine muscles compress the maternal radial arteries and veins of the placental bed, resulting in an obliteration of their lumina. Moreover, the strong myometrial contraction helps haemostasis by opposing the uterine walls firmly against one another and producing direct pressure on the placental site (Khan & El-Refaey 2006). Control of postpartum bleeding requires complete integration between the endocrine and coagulation systems.

2.2. Endocrine mechanisms of the third stage of labour

Similar to all muscular activity, uterine contractility requires both electrical and hormonal stimuli. Intrinsic activity of the uterine muscle may be mediated via stretch receptors which may involve both neural and neurohormonal mechanisms. There are two classes of natural hormones linked to the third stage uterine activity, namely oxytocin and prostaglandins.

2.2.1. Oxytocin

There is a large amount of clinical experience with therapeutic oxytocin in the induction of labour and prevention of PPH. However, the role of oxytocin in the mechanism of the third stage of labour is not well understood. Oxytocin is a nonapeptide hormone released by the posterior pituitary gland which acts on the female reproductive system to cause increased uterine contractions. It was the first peptide to be chemically synthesised to produce a biological active form. The oxytocin receptor is highly expressed in the endothelium, placenta, amnion and decidua. There is a strong increase in the density of oxytocin receptor in early labour. Its level rises up to 200 times that in the non-pregnant state (Gimpl & Fahrenholz 2001). A clear physiological relationship between oxytocin and the third stage of labour event has not yet been elucidated for many reasons. Firstly, oxytocin can be synthesised locally in a paracrine system operating in the amnion, chorion and

decidua. Therefore, a simple oxytocin assay is unreliable to reflect the exact concentrations of the hormone at the myometrium. Furthermore, there is no relationship between oxytocin plasma levels and the density of oxytocin receptors. Finally, the high activity of oxytocinase degrades the oxytocin before it reaches the target tissues. This also explains the non significant increase in plasma oxytocin concentration during labour (Gimpl & Fahrenholz 2001).

During labour, oxytocin is released in short duration discrete pulses (Fuchs et al., 1991). Thornton and his colleagues found that the oxytocin pulse does not occur at the same time as the uterine contraction. Some women with a completely normal third stage of labour do not experience an increase in oxytocin levels after the delivery of the baby. In addition, it is not necessary to have an oxytocin pulse in order to deliver the placenta (Thornton, Davison & Baylis 1988).

2.2.2. Prostaglandins

Prostaglandins are found in most tissue and organs including decidua, fetal membranes and placenta. They are involved in uterine contraction through paracrine and autocrine signals. Prostaglandins augment oxytocin induced contraction. Studies also suggest that prostaglandins increase the expression of the oxytocin receptors (Chan, Berezin & Daniel 1988). The presence of prostaglandins in maternal blood during labour is of interest because the sensitivity of uterine tissue to prostaglandins increases with the progress of gestation until it reaches a maximum at term. Prostaglandin is detected in the blood of women during labour before each uterine contraction, but very little is present at the end of the contraction. This indicate that prostaglandin is not released because of the contraction (Karim 1968).

The localisation of prostaglandin receptors in the tissue is important. The contraction promoting receptors EP1 and EP3 are found in the fundus while relaxation promoting receptors (EP2 and EP4) are more abundant in the cervix to facilitate the passage of the fetus during delivery (Olson 2003). It has been observed that large amount of prostaglandins are released during the third stage of labour. Plasma levels of prostaglandin $F_{2\alpha}$ reach a maximum level and started to decline within 10 minutes of placental separation, and return to pre-labour levels within 2 to 3 hours. This rapid

decline of plasma levels suggests that prostaglandin is mainly produced by cells at the placental site and fetal membranes. The latter is known to be the most important source of prostaglandins (Noort et al., 1989). This reflects the active role of prostaglandins in ensuring the haemostasis after labour.

2.3. Mechanism of coagulation

Coagulation at the placental site has an important role in the prevention of PPH. Around the time of delivery many changes both in the coagulation factors and fibrinolysis agents take place. There is consumption of platelets and blood coagulation factors including fibrinogen by clot formation and haemostasis at the placental site. This causes a decrease in the peripheral plasma fibrinogen levels. In a study performed by Bonnar and his colleagues, samples of uterine vein blood were obtained during caesarean sections. They found that the time of placental separation was accompanied by a striking activation of the local clotting mechanism and a sharp increase in factor VIII (Bonnar et al., 1970). In the peripheral circulation, there was a rapid increase in the clotting factors VIII and V and a decrease in plasma fibrinogen. Within one hour of delivery, fibrinolytic activity returns to its non-pregnant levels, and there is an increase in the level of fibrin/fibrinogen degradation products. In early puerperium, changes promoting coagulation take place. This includes an increase in the fibrinogen level and a swift increase in platelet count. The level of factor VIII remains high (Bonnar, McNicol & Douglas 1970). These changes may explain the increased susceptibility to thromboembolic complications during puerperium.

3. Aetiology and risk factors of PPH

The causes of PPH can be classified into 6 main categories (Table 7):

3.1. Uterine atony

The most common cause of PPH is the failure of uterus to contract (uterine atony). This pathology is responsible for 75-90% of primary PPH (Koh, Devendra & Tan 2009). Uterine atony occurs when the relaxed myometrium fails to contract and constrict the blood vessels. At term, about 600 ml of blood enters the uteroplacental circulation every minute (Rajan & Wing 2010). Therefore, over a short time of myometrial relaxation can lead to fatal haemorrhage. There are many risk factors associated with PPH (Table 7). However, uterine atony may occur in women without any risk factors. About 26% of women who underwent hysterectomy had no identifiable risk factors (Clark et al., 1984). In the UK, uterine atony was responsible for 53% of peripartum hysterectomy between February 2005 and February 2006 (Knight & Ukoss 2007). Although the most common risk factor is a previous history of PPH, there are many other important associated risk factors. Also, there may be more than one cause for the PPH and each cause requires a different treatment. Therefore, clinicians should be aware of all the risk factors so that they are able to anticipate and manage PPH in line with their own unit's protocol.

Uterine atony may occur as a result of uterine over-distension caused by polyhydramnios, multiple pregnancies or fetal macrosomia. Labour related problems such as a prolonged first or second stage of labour may also lead to uterine atony, but uterine dysfunction before onset of labour might result in a delay in all the three stages of labour, leading to PPH. Very rapid labour either natural or caused by induction or augmentation is thought to cause strong uterine contractions. These may lead to myometrial acidosis and contractile failure (Quenby et al., 2004).

Chorioaminonitis also impairs uterine contraction during the first and second stages of labour. This results in prolonged labour, which is a risk factor of PPH. Other risk factors may also include history of antepartum haemorrhage (placental abruption or praevia), obesity and an age of > 35 years (Breathnach & Geary 2009).

Table 7. Factors associated with postpartum haemorrhage (Oyelese & Ananth 2010)

Uterine atony *Labour-related causes:* Induction of labour Oxytocin use Precipitated labour Prolonged labour Chorioaminonitis *Uterine overdistension* Multiple pregnancies Polyhydraminos Placental abruption with large intrauterine clot Fetal macrosomia *Anaesthesias* General anaesthesia with inhaled agents	**Retained placenta and clots** **Coagulation disorders** *Disseminated intravascular coagulopathy* Placental abruption Liver dysfunction Amniotic fluid embolism Intrauterine fetal demise *Thrombocytopenia* *Inherited bleeding dysfunction* eg, von Willebrand disease *Anticoagulant therapy* **Uterine inversion**
Genital tract trauma *Iatrogenic* Caesarean delivery Forceps delivery Vacuum delivery Episiotomy *Spontaneous* Genital tract lacerations Uterine rupture	**Implantation of the placenta into the lower uterine segment** *Placenta praevia* *Placenta accreta*

3.2. Genital tract trauma

Traumatic causes of PPH, including genital tract laceration, uterine rupture and uterine inversion, account for about 20% of all primary PPH. Generally, if PPH is not due to uterine atony, genital tract trauma is likely to be the cause of severe bleeding. Trauma may result from laceration of the cervix, vagina sidewall, perineum and episiotomy, or from uterine rupture. Genital tract trauma could also be iatrogenic during caesarean delivery or instrumental delivery such as forceps or vacuum. These are risks for genital tract trauma and PPH should be highly anticipated.

3.3. Retained placenta and clots

Retained placenta or placental fragments, either as disrupted portions or more rarely a succenturiate lobe, may cause inadequate uterine contraction. Retained products of conception may act as a physical or endocrine block against strong uterine contraction which constricts the blood vessels at the placental bed. However, most cases of retained placenta are due to dysfunctional postpartum myometrial contraction (Oyelese & Ananth 2010).

3.4. Coagulation disorders

Both inherited and acquired coagulation defects are associated with excessive blood loss postpartum. Disseminated intravascular coagulation associated with abruption placenta (Figure 14), retained dead fetus, amniotic fluid embolism, excessive blood loss and massive blood transfusion may all lead to PPH. Other coagulation disorders such as thrombocytopenia, von Willebrand's disease and anticoagulant drugs also may cause massive blood loss postpartum.

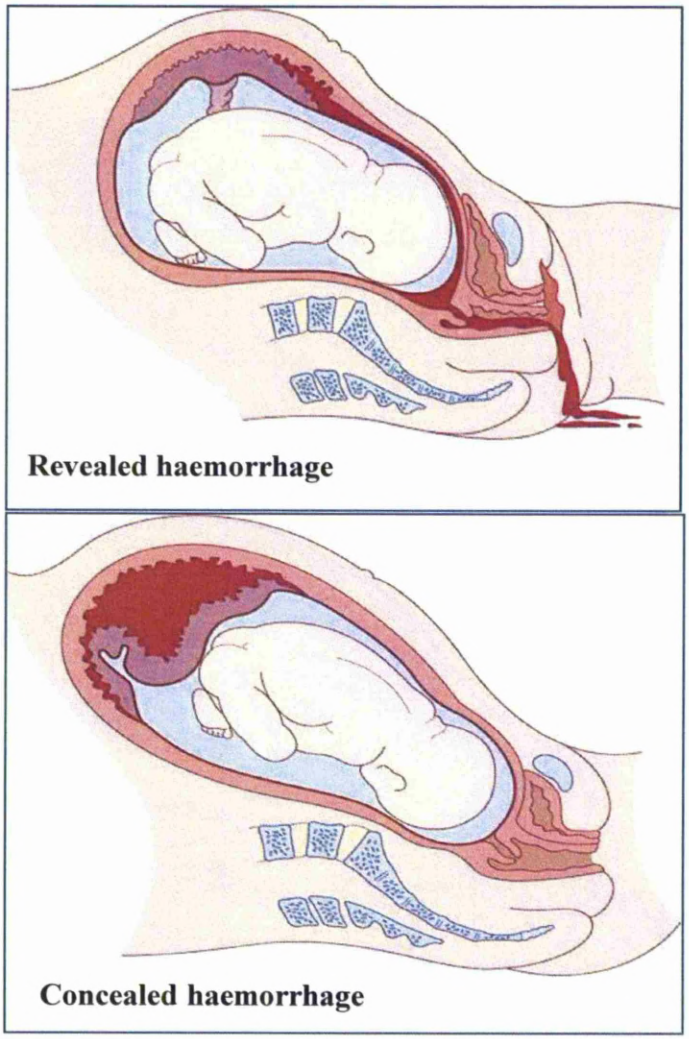

Figure 14. Abruptio placenta (premature separation of placenta). Two types of bleeding, revealed and concealed haemorrhage (Baker & Kenny 2011) (Obstetrics by Ten Teachers, 19th edition)

3.5. Uterine inversion

Uterine inversion is rare but life threatening condition. It occurs secondary to excessive cord traction during the delivery of the placenta. The resulting PPH is likely to be caused by a failure of the myometrium to contract and compress the blood vessels. The amount of shock may be inappropriate for the amount of blood loss. However, this cause of PPH can be fatal if not recognised and treated appropriately and quickly.

3.6. Implantation of the placenta into the lower uterine segment

The anatomical and physiological limitations of the lower uterine segment cause ineffective uterine contraction to prevent PPH after separation of the placenta from its bed. Implantation of the placenta in the lower segment (placenta praevia) makes haemorrhage much more likely (Figure 15). If the placenta invades the myometrium (placenta accreta), there is no clear cleavage line and attempts of removal can cause tearing of the placenta. The remaining fragments and opened sinuses will lead to severe haemorrhage.

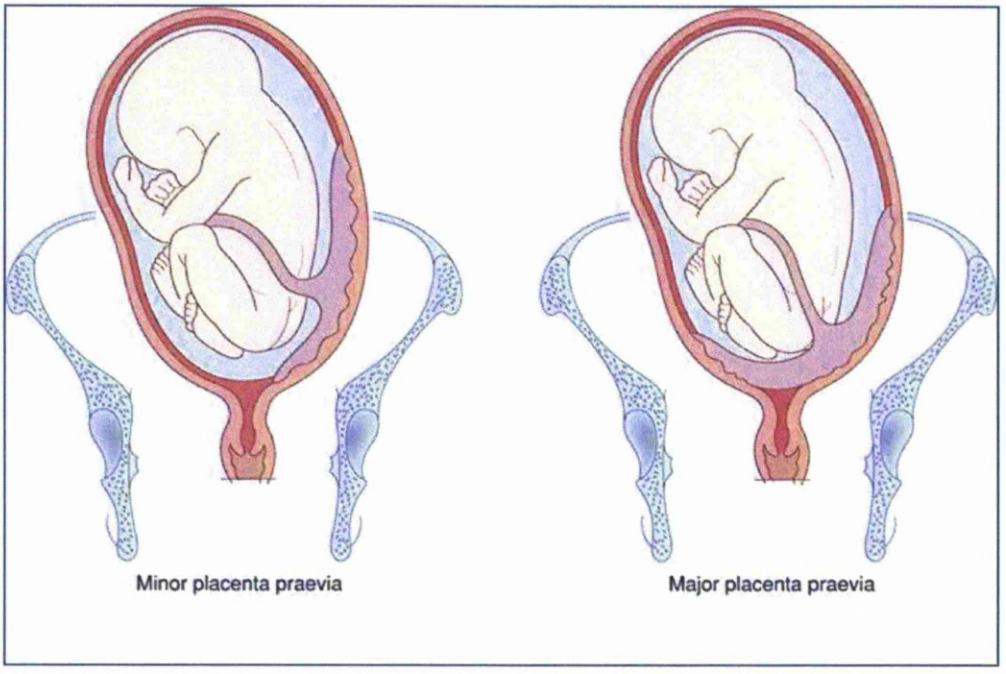

Figure 15. Placenta praevia (a) minor placenta praevia (b) major placenta praevia (Baker & Kenny 2011) (Obstetrics by Ten Teachers, 19th edition)

4. Management of Postpartum Haemorrhage

The majority of PPH occurs in women who do not have well-known risk factors. Therefore, the first step in reducing maternal morbidity and mortality of PPH is to provide preventive management for all women during labour. Each woman should be assessed for the likelihood of developing PPH before labour and staff need to be prepared to manage women who develop PPH. Appropriate precautions should be taken for all women who are at risk.

4.1. Prevention of Postpartum Haemorrhage

Methods for prevention of PPH have been developed and introduced to the intrapartum care to reduce maternal morbidity and mortality due to PPH. It mainly involves the active management of the third stage of labour (AMTSL), the main component of which is the use of uterotonics such as oxytocin, ergot alkaloids, prostaglandins and misoprostol.

4.1.1. Active versus expectant management of the third sage of labour

Third stage of labour or 'placental stage' is defined as the time from the delivery of the fetus until the delivery of the placenta and fetal membranes. The correct management of the third stage of labour plays an important role in the prevention of PPH. The physiological or the expectant management of the third stage of labour involves spontaneous delivery of placenta with maternal pushing efforts whereas active management involves a utrotonic plus other activities such as uterine massage, early cord clamping and cutting, or controlled traction on the umbilical cord and administration of uterotonics. This depends on different organisations' recommendations (Table 8). It is advised that for the first two hours after delivery, the uterus should be palpated to check that the uterus is well contracted with massage being performed as needed, making sure that the uterus does not become soft (relaxed) after stopping the massage (FIGO/ICM 2004a; Hofmeyr, Abdel-Aleem & Abdel-Aleem 2008).

Table 8. Recommendations for active management of third stage of labour (AMTSL) according to the organisation; CCT (controlled cord traction); FIGO, International Federation of Gynaecology and Obstetrics; ICM, International Confederation of Midwives; WHO, the World Health Organization; RCOG, Royal College of Obstetrics and Gynaecology; NICE, the National Institute for Health and Clinical Excellence; SOGC, The Society of Obstetricians and Gynaecologists of Canada

Organisation	Oxytocin	Cord clamping	Time of oxytocin	CCT	Uterine massage	Placental cord drainage
FIGO & ICM (FIGO/ICM 2004b)	Yes	–	Within one minute after delivery of baby	Yes	Yes After placental delivery	–
RCOG (RCOG 2009)	Yes - 5 IU or 10 IU by intramuscular injection	Early cord clamping only when indicated	–	–	–	–
NICE (NICE 2007)	Yes 10 IU IM	Early	–	Yes	–	–
WHO (WHO 2009)	Yes	Late (at around 3 minutes)	Within 1 minute after delivery of baby	Yes	Yes	–
SOGC (Leduc et al., 2010)	Yes 10 IU IM	Delayed	After delivery of the anterior shoulder	–		–

Initial trials of AMTSL found that the incidence of PPH was lower in actively managed women. The Bristol third stage trial compared active and expectant management in 1695 women. It found that expectant management was associated with a three times higher likelihood of PPH compared to the active management (OR 3.1, 95% CI 2.3-4.2) (Prendiville et al., 1988). The most recent Cochrane systematic review compared active versus expectant management of labour concluded that AMTSL in hospitals with mixed levels of PPH risk reduces the risk of PPH > 1000 ml (risk ratio (RR) 0.34, 95% CI 0.14 to 0.87), three studies, 4636 women and the incidence of blood transfusion. In the subgroup of women at low risk of excessive bleeding, there were similar findings (average RR 0.31, 95% CI 0.05 to 2.17, two studies, 2941 women), except there was no significant difference identified between groups for severe haemorrhage or maternal Hb less than 9 g/dl (at 24 to 72 hours). The review concluded that the active management also increases the risk of side effects such as postnatal hypertension, after pains and return to hospital due to bleeding. The trials included in this review used syntometrine or ergometrine in their intervention arm which could explain the occurrence of theses adverse outcomes. The AMTSL is also associated with a reduction in the birth weight which may reflect a reduction in the neonate blood volume due to early cord clamping (Begley et al., 2011).

The components of the AMTSL have been investigated to find out the effect of different practice on the reduction of postpartum blood loss and the possible adverse effects. These included the timing of administration of the uterotonics and the timing of cord clamping, uterine message and whether to apply cord traction for placental delivery or not. The administration of uterotonic prior to placental delivery raised the concerns that this could inhibit the delivery of the placenta. Available data propose that immediate administration of uterotonics does not lead to greater need for manual removal of placenta (Rajan & Wing 2010). However, the impact of early administration on blood loss remains uncertain. There was no difference in the incidence of PPH between women who had oxytocin immediately after the delivery and who had oxytocin after the delivery of placenta [56/745 (7.5%) vs. 72/741 (9.7%), P=0.15] (Jackson et al., 2001). Another small trial found that the rate of PPH was lower in the group who received oxytocin after the delivery of the placenta compared

to those women who received oxytocin after the delivery of the anterior shoulder [0/24 (0%) vs. 4/27 (14.8%), P=0.05]. However, the trial was terminated early at this small sample size due to lack of resources and this may make it under powered (Huh, Chelmow & Malone 2004). The most recent Cochrane review of the timing of prophylactic uterotonics found that timing of the administration of oxytocin either before or after the expulsion of the placenta did not have any significant effect on the rate of PPH, length of the third stage of labour and the rate of placental retention (Soltani, Hutchon & Poulose 2010). This review included a small number of trials and investigated only intravenous infusion of the oxytocin. Therefore, it recommended further studies to examine different routes of administration and different maternal and neonatal outcomes.

Only one study addressed the subject of the uterine massage. The 200 subjects were randomised to receive routine active management of labour (oxytocin 10 IU I.V. or I.M, immediate cord clamping and controlled cord traction) with uterine massage every 10 minutes for the first 60 minutes or active management without uterine massage. The trial demonstrated a significant reduction in blood loss by 78 ml and 80 % reduction in the need for additional uterotonics in the massage group compared to the non-massage group. There was no significant reduction in the rate of PPH (4/98 (5%) vs., 8/102 (7%) (Abdel-Aleem et al., 2006). A larger study is needed to confirm the finding and to show the effect of uterine massage in settings where injectable uterotonics are available. This would be of great importance in low resource setting where PPH is the main cause of maternal mortality.

Early umbilical cord clamping after birth is one component of the triad that makes up the original description of the active management of labour. However, the latest Cochrane review suggested that delayed cord clamping seems not to increase the incidence of PPH and may be beneficial to the baby in terms of promoting better iron stores, although fewer infants in the early cord clamping group require phototherapy for jaundice compared to the late cord clamping group (RR 0.59, 95% CI 0.38-0.92) (McDonald & Middleton 2008). A recent study to investigate the effects of delayed umbilical cord clamping compared with early clamping on infant iron status at 4 months of age in a European setting showed a 45% (95% CI 23-71%) higher mean

ferritin concentration (117 μg/L v 81 μg/L, P<0.001) and a lower prevalence of iron deficiency (1 (0.6%) v 10 (5.7%), P=0.01) (Andersson et al., 2011).

Another concern in the protocol of the management of the third stage of labour is the risk of uterine inversion with cord traction by untrained attendants. Therefore, the current WHO statement on PPH recommended that skilled birth attendants should offer AMTSL to all women; it does not advocate active management by non skilled attendants (WHO 2007). The International Federation of Gynaecologists and Obstetricians (FIGO) and International Confederation of Midwives (ICM) joint statement in 2003 stated that active management should be routinely offered to all women in the third stage of labour providing that all birth attendants should have the knowledge and the proper facilities to perform the active management safely (FIGO/ICM 2004a). According to this statement, the active management of labour consists of the use of uterotonics (preferably oxytocin), controlled cord traction and uterine massage. However, a recent multicentre randomised controlled trial showed that omission of controlled cord traction has very little effect on the risk of severe haemorrhage (Gulmezoglu et al., 2012).

Until recently, the individual components of the third stage of labour had not been examined extensively in research. Therefore, there has been confusion about the optimal management protocol. A survey of European policies of the management of the third stage of labour revealed a considerable variation within and between countries in the choice and timing of the uterotonic administration as well as cord clamping (Winter et al., 2007). More research is needed to examine the effect of each component of the third stage of labour and the proper uterotonics for the active management in the developing countries where the potential risk of PPH is high.

4.1.2. Oxytocin for the third stage of labour

Oxytocin is a naturally occurring uterotonic produced by the posterior lobe of the pituitary gland. It was synthesised in 1953 by Du Vigneaud and colleagues (Du Vigneaud, Ressler & Trippett 1953). Its mode of action involves the stimulation of the upper uterine segment to contract in a rhythmic manner. Oxytocin has a rapid onset of action and short half life (mean 3 minutes) when given IV. Therefore, a continuous

infusion is needed to maintain uterine contraction. By contrast, use of the intramuscular route results in a slower onset of action (3-7 minutes) but a longer duration of action (up to 60 minutes). Oxytocin has anti-diuretic effect; therefore, prolonged infusion can cause water intoxication particularly if given in large volume of electrolyte-free solution. Bolus intravenous should be used with caution as oxytocin results in relaxation of vascular smooth muscles and may cause profound hypotension (ACOG 2006; Breathnach & Geary 2009).

A Cochrane review included over 3000 women in seven trials with considerable variation in the doses and the route of administration found a clear benefit of the use of oxytocin in the third stage of labour compared to no uterotonic. Oxytocin use was associated with a lower incidence of PPH > 500 ml (RR 0.5, 95% CI 0.43-0.95) and > 1000 ml (RR 0.61, 95% CI 0.44-0.87) and less need for therapeutic uterotonics (RR 0.5, 95% CI 0.39-0.64) (Cotter, Ness & Tolosa 2001). Compared to ergot alkaloids, there was no difference in the rate of PPH > 500 ml and the need of additional uterotonics between these two uterotonics. However, oxytocin administration was associated with less frequent manual removal of placenta (RR 0.57, 95% CI 0.41-0.79) and less raised blood pressure (RR 0.53, 95% CI 0.19- 1.52), compared to ergot alkaloids (Cotter, Ness & Tolosa 2001).

A new delivery system of oxytocin using a prefilled oxytocin injection (Uniject) is of great importance in the prevention of PPH particularly in less developed countries. A study conducted in Angola found that Uniject showed marked reduction in the rate of blood loss ≥ 500 ml compared to no treatment [316/782 (40.4%) vs. 67/814 (8.2%), P<0.001] (Strand et al., 2005). The midwives in a study conducted in Indonesia found the device easier and more practical than the traditional way using needle, syringe and ampule (Tsu et al., 2003). These trials finding suggest that the introduction of the Uniject may facilitate the application of the AMTSL in the developing countries particularly in places with high incidence of HIV and viral hepatitis.

4.1.3. Ergot alkaloids for the third stage of labour

Ergometrine results in prolonged and sustained uterine contractions in both the upper and the lower uterine segments via an increase in the calcium influx which in turn

activates the uterine smooth muscles to contract in a tetanic manner. The onset of action is 2-5 minutes after intramuscular injection of the standard dose (0.25 mg of ergometrine). It is mean plasma half life is 30 minutes and the clinical effect continues for 3 hours. The administration is mainly by intramuscular route, although oral and intravenous routes are also possible. Ergot alkaloids mediate its action via stimulation of the α-adrenergic receptors which can results in vasoconstriction and cause hypertension. Therefore, it is contraindicated in women with hypertension, pre-eclampsia, heart disease, migraine, Raynaud's syndrome and peripheral vascular disease. The most commonly reported side effects are nausea, vomiting, headache, dizziness and increased blood pressure (Breathnach & Geary 2009).

For the AMTSL, ergot alkaloids were compared to no uterotonic agent in a systematic review for the Cochrane collaboration. Parenteral administration of ergot alkaloids significantly decreased the mean blood loss and the risk of PPH > 500 ml (RR 0.38, 95% CI 0.21-0.69). On the other hand, the treated women had elevated blood pressure (RR 2.60, 95% CI 1.03-6.57), vomiting (RR 11.81, 95% CI 1.78- 78.28) and after pain requiring analgesia (RR 2.53, 95% CI 1.34-4.78) (Liabsuetrakul et al., 2007). Most studies used intramuscular ergometrine. However, the one study that used the intravenous route reported that 0.5 mg intravenous ergometrine was associated with a significant increase in the incidence of manual removal of placenta (3%) compared to the physiological management of the third stage of labour (0.1%) (Begley 1990) . The one trial that examined the oral route showed no significant benefit of ergot alkaloid over placebo and concluded therefore that oral ergometrine is not an alternative to oxytocin in the management of the third stage of labour (De Groot 1996). Overall the evidence suggests that although ergometrine is effective at PPH prophylaxis, oxytocin is much better tolerated.

Syntometrine® is one of the most widely used uterotonics in the UK. It is combination of 5 IU oxytocin and 0.5 mg ergometrine. It has both the advantages of the oxytocin rapid onset of action and the sustained uterine contractility provided by the ergometrine. The Cochrane review of the prophylactic use of syntometrine versus oxytocin found that the syntometrine reduced the occurrence of PPH > 500 ml [OR 0.82, 95% CI 0.71 to 0.95] but not of PPH > 1000 ml (OR 0.78, 95% CI 0.59-1.04).

Syntometrine was associated with significantly more risk of side effects such as hypertension [OR 2.81 (95% 1.17 to 6.73)] and vomiting (OR 4.92, 95% CI 4.03-6.00) (McDonald, Abbott & Higgins 2004). Therefore, although oxytocin would appear to be marginally less effective than syntometrine, it is generally recommended as first line treatment due to its low rate of side effects and greater stability to light and heat.

4.1.4. Oxytocin agonist

Carbetocin is a synthetic long acting analogue of the natural human oxytocin. It binds to the oxytocin receptor on the uterine muscle and produce rhythmic contraction by releasing calcium ions. Carbetocin was produced by modifying the oxytocin molecule and this prolonged its half life and reduced its enzymatic degradation. Carbetocin has a similar rapid onset of action, the same side effect profile as oxytocin, and can be administered intramuscularly or intravenously. Intravenous carbetocin has a half life of approximately 40 minutes (Sweeney et al., 1990). The uterine effects of the intramuscular injection persist for approximately 120 minutes and for the intravenous injection for about 60 minutes (Hunter, Schulz & Wassenaar 1992). The optimal dose of carbetocin is 100 mcg intravenous or intramuscular (van Dongen et al., 1998). The efficacy of carbetocin in the AMTSL has been investigated after vaginal and after caesarean delivery. A randomised, double blind, placebo controlled trial found that single intramuscular injection of 100 mcg carbetocin was more likely to prevent PPH in high risk women than a 2 hour intravenous infusion of 10 IU oxytocin. The main difference was observed in the need of additional interventions either additional uterotonic or uterine massage. An additional intervention was required in 44.6% of the women who received carbetocin and in 63.6% of women who were managed with oxytocin ($P<0.025$). However, no significant differences were observed for other efficacy variables which included change in haemoglobin and haematocrit over the initial 24 hours postpartum, estimated blood loss, uterine tone and the amount and type of lochia (Boucher et al., 2004). Another RCT which investigated the efficacy of intramuscular carbetocin (100 mcg) versus intramuscular syntometrine in the prevention of PPH did not demonstrate a significant difference between the two treatment arms in terms of reduction in the haemoglobin level, need for additional

uterotonics, rate of PPH > 500 ml and the incidence of retained placenta (Leung et al., 2006). The use of carbetocin was associated with lower rates of nausea, vomiting and increased blood pressure, but a higher incidence of maternal tachycardia (Leung et al., 2006). Carbetocin is currently approved in 23 countries as single intravenous injection for the prevention of uterine atony after caesarean section under epidural or spinal anaesthesia (Peters & Duvekot 2009; Rath 2009). Data from 2 RCTs were evaluated in Cochrane meta-analysis to determine the efficacy of carbetocin in caesarean delivery. The risk of PPH > 500 ml was not significantly decreased in the women who were treated with carbetocin compared with the oxytocin group (RR 0.71, 95% CI (0.14-3.35). However, carbetocin significantly decreased the need for additional uterotonics (RR 0.44, 95% CI (0.25-0.78) and uterine massage (RR 0.38, 95% CI (0.18-0.8) compared with oxytocin. This review concluded that most of the outcomes were extracted from a single trial and the available data on carbetocin has several limitations as most of the trials did not use blood loss as a primary outcome (Su, Chong & Samuel 2007). Therefore, that evidence of carbetocin superiority over the currently available uterotonic is not yet proven and it remains outside of most international PPH guidelines.

4.1.5. Prostaglandins E and F for the third stage of labour

Prostaglandins play a major role in producing uterine contractions. The exogenous administration of different types of prostaglandin has been investigated for prevention and treatment of PPH. Many prostaglandins and its analogues have been available for these indications. They include prostaglandin F2α (dinoprost) and its analogue (carboprost), prostaglandin E_2 analogue (dinoprostone, sulprostone) and prostaglandin E_1 analogues (misoprostol and gemeprost). This section will focus on all the above mentioned prostaglandins except misoprostol which will be described in the following section as extensive research has been conducted to find out the effectiveness of misoprostol in the AMTSL particularly in the developing world.

Prostaglandin F2α and prostaglandin E are mainly used for the treatment of PPH and the data of their application for PPH prophylaxis is very limited. Ten small trials involving injectable prostaglandins have been evaluated in the Cochrane review for prostaglandins for preventing PPH. When compared with no uterotonic, injectable

prostaglandins had less mean blood loss and also it has less blood loss and shorter duration of the third stage of labour compared to conventional uterotonics (Gulmezoglu et al., 2007). However, a higher incidence of abdominal pain, vomiting and diarrhoea has been reported with the injectable prostaglandins. The use of one prostaglandin E_2 analogue (sulprostone) was associated with cardiac arrest in 3 women and it was therefore voluntarily withdrawn by the manufactures (Sharma & El-Refaey 2003). Overall, injectable prostaglandins appear to be superior to placebo but were not preferable to conventional uterotonics in the management of the third stage of labour (Gulmezoglu et al., 2007).

4.1.6. Misoprostol for the third stage of labour

Overall, Misoprostol appears to be less effective than conventional uterotonics. But, it is clear that misoprostol is better than placebo for AMTSL. Data from studies about misoprostol for PPH is difficult to interpret as most of them were un-blinded and subjective measurement of blood loss is difficult and inaccurate. More details was mentioned in the first section of this chapter.

4.2. Treatment of Postpartum Haemorrhage

The management of PPH should be started antepartum and intrapartum. The likelihood of a woman developing PPH should be assessed carefully and appropriate precautions should be taken in women who are at risk. All women at risk should have intravenous access and have appropriate uterotonics and blood products available.

A systematic approach should be taken when managing women presenting with heavy bleeding after delivery. The women should be assessed carefully to find out the cause of bleeding and they should be resuscitated if develop signs and symptoms of haemorrhagic shock. The first action should be to call for help and to alert an experienced obstetrician, followed by resuscitation of the patient, assessment of the uterine tone and rule out the possibility of retained products of conception, genital tract trauma and coagulopathy (commonly known as the 4Ts: tone, tissue, trauma, thrombin). Bladder evacuation and keeping a catheter in place is very important as a distended bladder could be the only cause of uterine atony and PPH. The second line

of treatment, if atony persists is bimanual uterine compression (Figure 16) which should be applied and additional uterotonics administered immediately. If bleeding persists despite this, exploration under anaesthesia should be done before transition to surgical management (RCOG 2009).

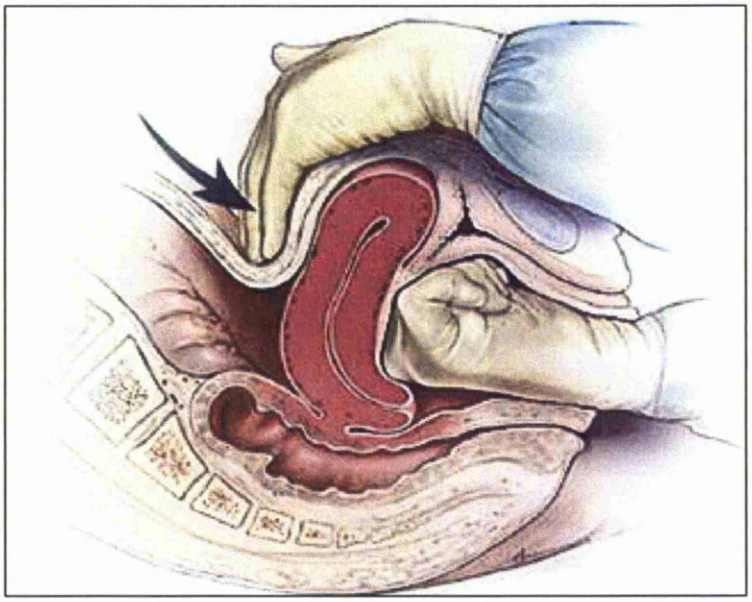

Figure 16. Bimanual uterine massage for management of uterine atony (Anderson & Etches 2007)

4.2.1. Medical treatment of PPH

The first line medical treatment of uterine atony involves administration of oxytocin or ergometrine as a bolus doses. Despite extensive and extended use of injectable uterotonics over the last few decades, there were no clinical trials comparing oxytocin and ergometrine as first line therapies for treatment of uterine atony. Although both agents seem to be equally effective, oxytocin is more widely used as it has fewer side effects and can be easily used in women with hypertension and preeclampsia. On the other hand, intravenous administration of 5 IU oxytocin can result in profound hypotension due decrease in the resistance of vascular smooth muscles (Figure 17) (Weeks 2010). Therefore, it is recommended to use 5 IU oxytocin as slow intravenous injection and replace it with a continuous infusion of 20 IU oxytocin in 500 ml crystalloid solution to maintain uterine contraction if bleeding persists. Ergometrine can be administered as 0.25 mg intramuscular injection. It is contraindicated in women with hypertension and causes nausea, vomiting and dizziness.

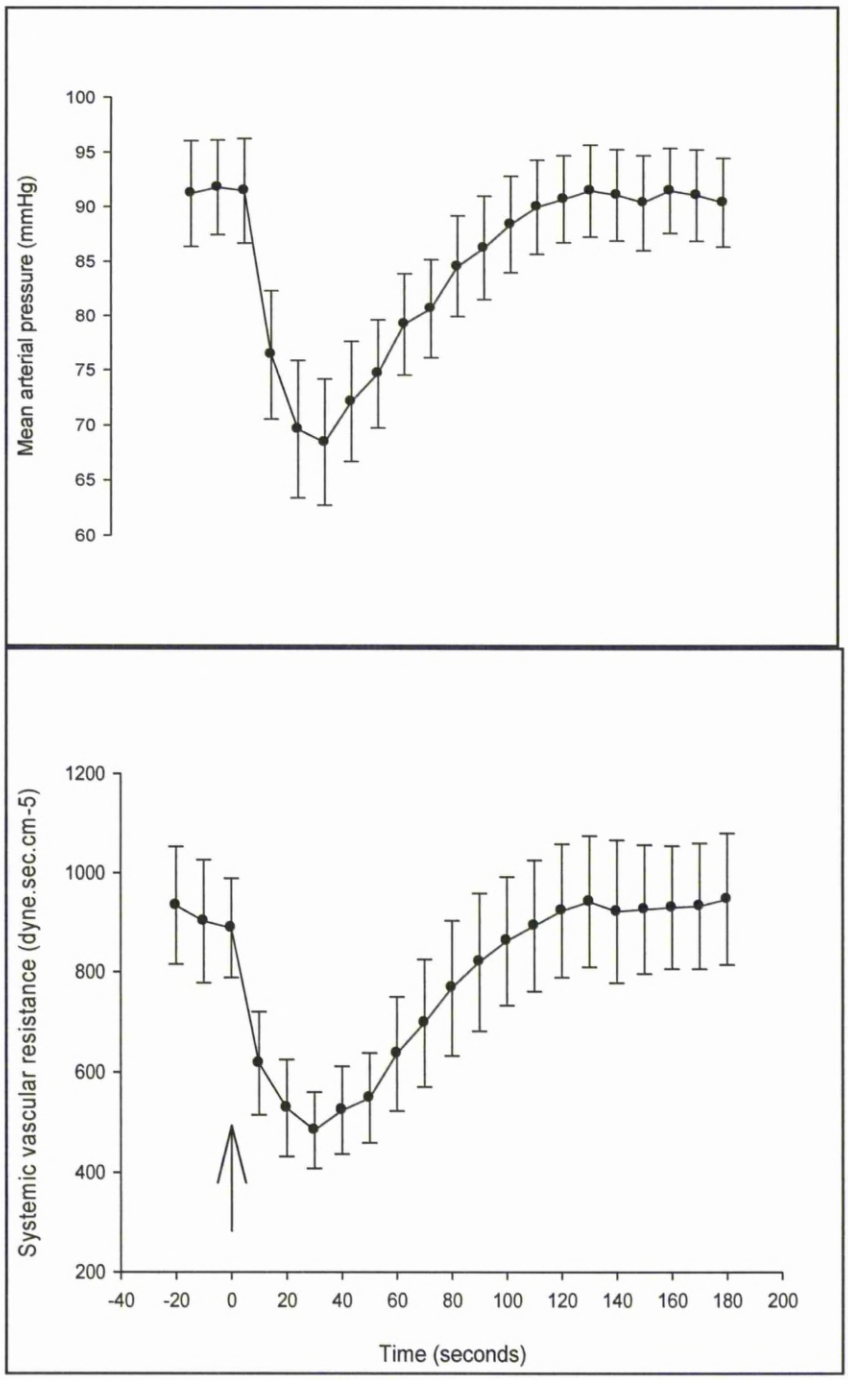

Figure 17. Systemic vascular resistance, mean arterial pressure and cardiac output represented as means (95% confidence intervals) vs. time (seconds). Arrow indicates timing of 5 unit oxytocin bolus (from Weeks 2010 with permission)

Carboprost (PGF2α) is considered as the second line for the management of PPH in a uterus unresponsive to oxytocin or ergometrine. However, there were no trials comparing it with other uterotonics. It has a longer duration of action than dinoprostone (PGE_2). Carboprost can be given as a deep intramuscular or direct intramyometerial injection. The latter route has a rapid onset of action and can be achieved under direct vision during caesarean section, transabdominally or transvaginally. The peak plasma concentration of the intramuscular route is achieved at 15 minutes and at less than 5 minutes for the intramyometerial injection. It is available in single dose vials of 0.25 mg. The dose can be repeated every 15 minutes up to a maximum of 2 mg (8 doses) (Breathnach & Geary 2009).

Misoprostol is generally recommended as the last line of management of uterine atony in hospital sittings and in the presence of other injectable uterotonics. Also, misoprostol could be an appropriate alternative when parenteral prostaglandin is not available or when it is contraindicated (usually asthma). The authors of the latest Cochrane review for treatment of PPH concluded that the available evidence was not enough to support the use of misoprostol instead of oxytocin and ergometrine as a first line treatment of PPH (Mousa & Alfirevic 2007). The use of misoprostol for treatment of PPH was discussed in more details in Section I.

4.2.2. Fluid and blood product for treatment of PPH

While investigating the aetiology of PPH and treating the cause, resuscitation and volume replacement should immediately take place. Rapid infusion with colloid-crystalloid solutions should be started. The haematologist and blood transfusion laboratory should be notified. Anaesthesia should be also notified to be ready for surgical interventions once needed. Baseline investigation should be ordered such as complete blood count as well as prothrombin time (PT) and partial thromboplastin time (PTT) (RCOG 2009).

4.2.3. Uterine tamponade

There are several methods based on tamponade to terminate uterine bleeding. These methods can include the most basic methods of uterine backing using long gauze

placed in the uterine cavity, or inserting a condom attached to the end of Foley catheter, a technique that is particularly useful in the developing countries. The one disadvantage of these devices is that they may mask uterine bleeding by blocking the outflow tract. Therefore, it is important to note the fundal levels at the time of packing (Rajan & Wing 2010).

A more developed balloon device such as Sengstaken-Blakemore tube, Rusch balloon and Bakri balloon have been used in Europe (Figure 18, (Georgiou 2009). These methods, except the Rusch balloon, have the advantage of providing an outflow tract for the continuous bleeding (Majumdar et al., 2010). The effectiveness of different tamponade methods has only been described in cases series since randomised controlled trials are unlikely to be conducted as the cases which need uterine tamponade is relatively rare. Uterine balloons have proved very popular with reported a success rate of 81% for the Sengstaken-Blackemore tube (Doumouchtsis et al., 2008) and 59% success for Rusch balloon (Majumdar et al., 2010). Therefore, uterine balloon tamponade is considered as important weapon in the treatment of PPH.

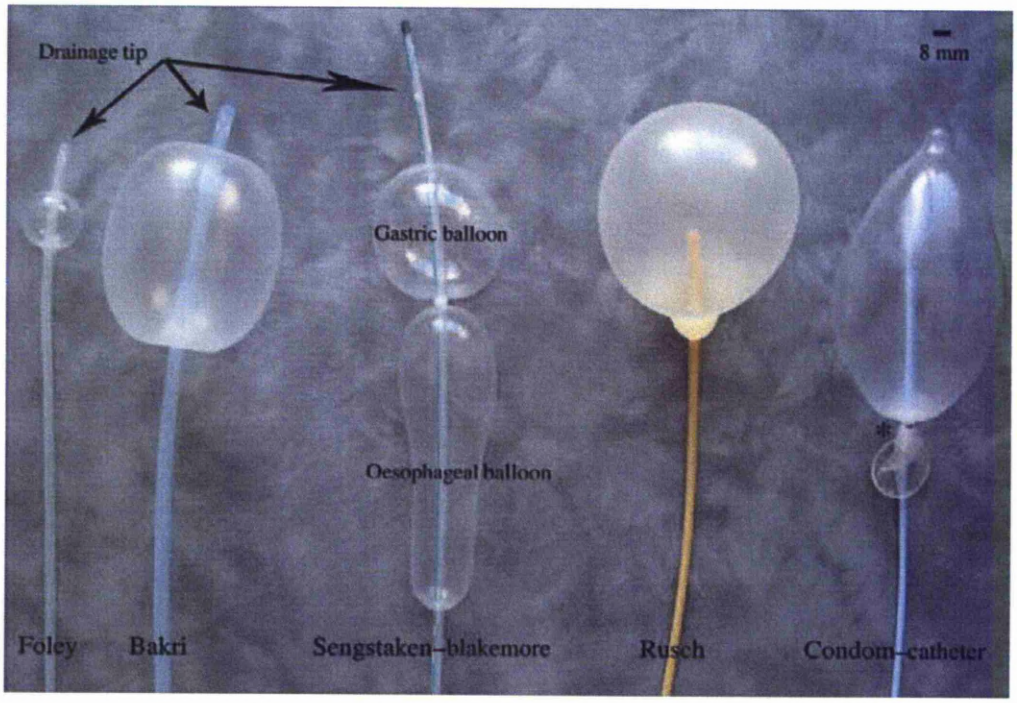

Figure 18. Distal component of tamponade balloons (Georgiou 2009)

4.2.4. Uterine compression and selective pelvic vessels embolisation

If bleeding continues despite all the above measures, operative intervention should follow quickly. This involves exploratory laparotomy via a midline vertical incision. Many compression methods have been described to control the uterine bleeding such as B-Lynch technique and through- and through square sutures. Some clinicians go immediately for hysterectomy as a life-saving procedure. The surgical management depends mainly on the health facilities and the experience of surgeons where the cases of PPH are treated. The B-Lynch suture was first described in 1997 by Christopher B-Lynch and modified techniques have evolved later (B-Lynch et al., 1997). The efficacy of uterine compression suture is high as demonstrated in several systematic reviews of cases series of women with severe PPH (Fotopoulou & Dudenhausen 2010; Ouahba et al., 2007).

Vascular embolisation technique can also be applied in haemodynamically stable patients with continued bleeding in centres with good facilities and trained personnel. It is a less invasive intervention and involves insertion of a catheter into the femoral artery to the aorta then to the uterine arteries. A success rate of 95-100% has been reported (Hansch et al., 1999; Vegas et al., 2006; Winograd 2008). Therefore, this technique is highly recommended as first line before surgical intervention. However, the facility to perform this technique is not widely available even in the developed countries.

5. Postpartum intrauterine pressure measurements of myometrial activity

Intrauterine pressure (IUP) measurements were introduced into clinical practice mainly to study uterine activity during labour. It assisted in the diagnoses of various labour dysfunctions such as obstructed labour, arrested labour and uterine hyperstimulation particularly during the induction of labour using uterotonics.The intrauterine pressure measurements have also been used extensively in research to investigate the uterine activity at different stages of the female reproductive life. This included studies of the uterine muscle activity during menstruation to investigate dysmenorrhoea (Lumsden, Kelly & Baird 1983; Milsom & Andersch 1985), studies of uterine stimulation during different procedures and stages of assisted reproduction (Bulletti & De Ziegler 2005) and research into uterine activity during the third stage of labour to explore the causes and treatment of uterine atony (Chong et al., 2001).

5.1. History of intrauterine pressure measurements

The current methods of intrauterine pressure measurements came after more than a century of evolving and improving techniques for this purpose. The first recording of intrauterine pressure was done by Heinricius in 1889. The catheter was a large balloon connected to a manometer by an electroplated catheter. The insertion of this large balloon required anaesthesia and produced marked uterine irritability. Therefore, efforts were directed to decrease the balloon size. In 1893, Westermark developed a smaller balloon tipped cannula which made the insertion easier and produced more accurate information (Smith 1984).

Although many modifications were applied to methods of the uterine measurements after this, the balloon tipped catheters remained the standard until the concept of the intrauterine pressure transducer was introduced in 1944. By moving the pressure sensor into the uterus, the inaccuracies caused by the transmission of the pressure wave to an external manometer was avoided. In 1962, improvements were made to the transducer which became more sensitive and well tolerated by the patients, and no longer severely restricted the woman's position and movement. By the early 1970's,

micro-transducers were developed and were being used for the evaluation of the uterine pressure during labour (Smith 1984).

5.1.1. Units for evaluating uterine activity

For the assessment of uterine muscle activity, we need to know how strong the contractions are, how frequent they are and how long they last. Therefore, ideally one would have one measurement or unit to describe all three factors which would provide a clear idea about the nature and the quality of the uterine contractions during labour. Several units have been introduced to assess uterine contractions such as Montevideo unit (MVU), Alexandria units (AU), area under the uterine pressure curve, active pressure integral (API), and mean active pressure (MAV). Although each method has its advantages and disadvantages, all have been used in practice and in research. The MVU is calculated by measuring the peak intrauterine pressure during the contraction and multiply them by the number of the contraction over 10 minutes. Many authors have pointed out that the MVU ignores the contribution of the duration of contractions and is also insensitive to the shape of the contractions. Although the Alexandria unit included the duration of the uterine contraction in its formula, it has not gained popularity. The use of 'area under the uterine pressure curves' takes into account the frequency, intensity and duration of the uterine contraction. However, it fails to differentiate between the active contraction and baseline tone. API is an integral of active pressure which is the intrauterine pressure minus the baseline pressure over a period of 15 minutes. Dividing the active pressure integral by the number of seconds in 15 minutes (900 sec) gives the mean of active pressure (MAP) (Phillips & Calder 1987).

5.2. Current methods of IUP measurement (Types of catheters)

There are different types of the intrauterine pressure catheters with variable shapes and design. Some of the catheters have a transducer at the tip (e.g. the Intran catheter) and some have a sensor at the tip connected to an external transducer (e.g. the Koala catheter; Figure 19 ; (Dowdle 1997). The catheter with a pressure transducer at the tip was introduced to overcome the disadvantages of the fluid filled polythene catheter which may cause uterine perforation. Partial blockage of the system with vernix or

blood clots also commonly leads to an underestimation of the uterine activity. This could result in clinicians giving an excessive amount of oxytocin and causing uterine hyperstimulation. Also, an increased incidence of endometritis has been reported (Steer et al., 1978). It has also been shown that transducer-tipped IUP catheters may artifactually elevate the basal uterine tone reading due to the physical mechanics inherent in the tipped transducer catheter. This may result in difficulties in the management of dysfunctional labour, augment or induced labour and in the diagnosis of abruptio placenta (Ross & Walton 1994).

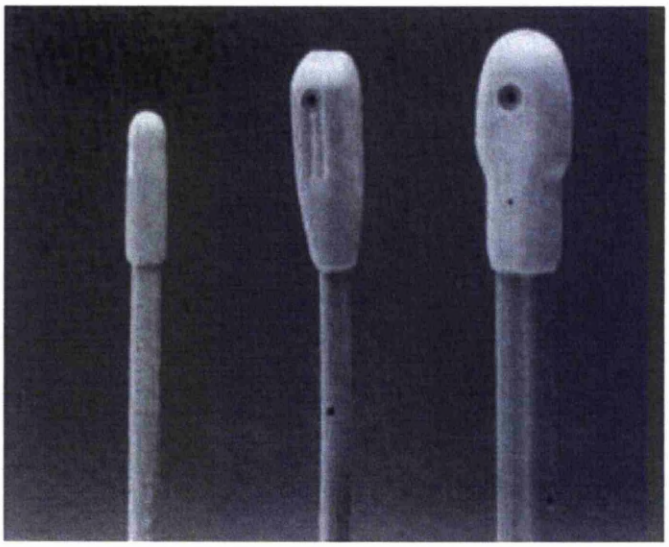

Figure 19. Koala catheter tip (left) as compared to the catheters with transducers at their tips: the Intran Plus (center) and SoftTrans (right) (Dowdle 1997)

The recently produced intrauterine pressure catheter with a sensor at the tip and an external transducer (Koala) shows some advantages over the catheter tip transducer (Intran). These include an easier insertion which occurs because the catheter has a softer, smaller catheter tip and a flexible body. It also gives a more accurate measurement as it has multiple holes to transmit all the amniotic pressure to the transducer. Also, unlike the transducer tipped catheters, the accuracy of the

measurements are not affected by changes in the temperature from body to room temperature, contact pressure or force as the transducer is located outside the body. It is also easier to set up and easier to zero while it is *in-utero* as the transducer is connected to an external reusable connector (Dowdle 1997).

The most recent development of the IUPC was the addition of a sensing membrane to the tip of the external transducer catheter. This new catheter was produced by clinical innovations and is known as the Koala IUP-5000E catheter (Figure 20). It employs air-coupling technology from a distally mounted flexible balloon that communicates through a sealed micro-tube to an externally located electronic reusable transducer in the monitor cable and connector. The purpose of this is to eliminate the direct tissue contact force erro.

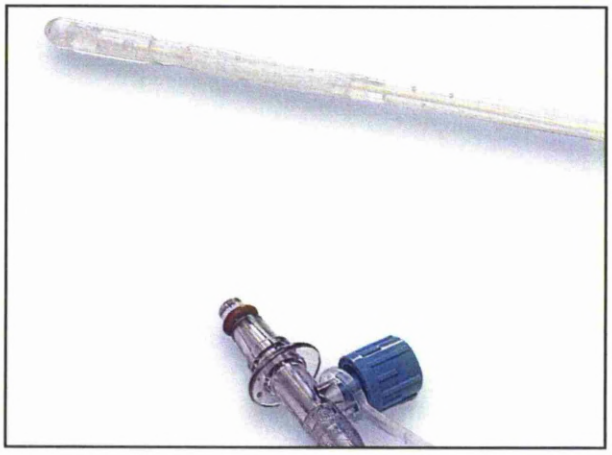

Figure 20. Koala IUP-5000E catheter (Clinical innovations)

5.3. The reliability studies of IUP catheters

Several studies have been conducted to investigate the reliability of IUPs during different stages of labour. Some of the studies used an in vitro uterine model to mimic several conditions during labour whereas others conducted the reliability test in a real situation during labour. One study used an in vitro uterine model to test the reliability of two catheters from Utah Medical. One was the sensor tipped transducer catheter and the other was fluid-filled catheter with an external transducer. In this model

several situations were introduced such as kinking of the catheter tip, using thick fluid to simulate thick meconium and using petroleum jelly on the catheter tip to simulate vernix obstruction. Both types of catheter showed satisfactory recording of the intrauterine pressure except when in the presence of simulated thick meconium. The study showed a significant decrease in peak pressures in fluid-filled catheter series at all pressure levels compared with the sensor-tip catheter series. Flushing of the fluid-filled catheter resulted in non-significant difference in the resting and peak pressures and the pressure waveforms between both types of catheters. Therefore, in presence of thick meconium it is advised to use a sensor-tip catheter to avoid the need for continuous flushing of the fluid-filled catheter (Devoe, Smith & Stoker 1993).

The transducer tipped catheter has been evaluated during the first and the third stages of labour. During the first stage of labour, the intrauterine pressure transducer-tipped catheter was evaluated clinically in 100 patients. In the study, the clinicians were able to successfully insert the catheter 95% of the time and there were no significant maternal or fetal complications. The time required for insertion was short and could be done without assistance. This type of catheter also needed few or no ongoing maintenance (Strong & Paul 1989). The accuracy of tip transducer catheter was investigated by introducing two catheters into the same amniotic fluid pocket and introducing two catheters into two different pockets. The study found that the information on the cumulative intrauterine pressure was reliable whenever the catheter's tip was located in the uterus (Chua et al., 1992).

Another study compared the IUP recorded from 3 different types of catheter during the first stage of labour. This included conventional fluid-filled catheter (Soft-Trans), catheter tip transducer (Intran plus) and external transducer catheter (Koala). The data showed a significant difference between Koala and the other catheters in ease of insertion and use and in level of clinical accuracy. However, it showed no significant difference between the catheters in safety during insertion, signal zeroing and signal drift (Dowdle 1997). The reliability of the air-charged coupled Koala catheter was compared with an electronic pressure transducer tipped catheter (Intran) during labour. Both catheters were bound together at their tips and introduced into the uterus. The data showed similar mean baseline tone, peak pressures, contractions frequency

and duration during labour. However, there was no reliability study for koala catheter during the third stage of labour (Dowdle 2003).

During the third stage of labour, the reliability of the transducer tipped catheter was evaluated at different locations in the uterus using two catheters tied together and two separate catheters. Whether the catheters were separate or tied, the comparison of individual active pressure readings revealed good agreement and the cumulative active pressure was very similar in each catheter in the same uterus (Chua et al., 1998). In these studies there was no gold standard to enable an assessment of accuracy.

Intrauterine pressure catheters have a wide range of applications in practice and research. Clinically, they are mainly utilised to assess the strength of uterine contractions during the first stage of labour and can help to diagnose labour dysfunction due to inappropriate uterine contractions' strength and frequency (Bakker, Van Rijswijk & van Geijn 2007).

In research, intrauterine pressure catheters have been used to study the uterine activity during menstruation to investigate the causes of dysmenorrhoea and also to explore uterine contraction during IVF procedures. Research into drugs' effect on uterine activity either during labour or postpartum, has utilised the intrauterine pressure catheters to measure this effect postpartum as it does not involve the fetus. Even though calcium channel blockers such as nifedipine and isradipine are mainly used in obstetrics to inhibit uterine contraction in preterm labour, many studies have investigated the relaxant effect of these drugs on uterine contraction during the third stage of labour (Ingemarsson et al., 1989).

Research into PPH has used intrauterine pressure catheters to measure uterine contractions during the third stage of labour. Early studies showed that medications such as oxytocin, ergometrine and prostaglandins all have a strong contractile effect on the uterine muscle. Intrauterine pressure catheters were widely used to investigate the effect of these medications and to compare different doses and routes for prevention and treatment of PPH.

Chapter 2

The risk of fever after administration of misoprostol for prevention of postpartum haemorrhage: systematic review and meta-analysis

1. Introduction

Misoprostol, a PGE_1 analogue, has been recommended as an alternative to parental uterotonics for the management of PPH because of its potency, ease of administration, stability at ambient temperature and cost effectiveness (Mousa & Alfirevic 2007). However, elevated body temperatures after its use in relatively high doses for PPH have raised concerns about its safety (Hofmeyr & Gulmezoglu 2008). Identification of the correct dosage is however difficult and recommended misoprostol dosages vary widely according to the route of administration, indication and gestation (Elati & Weeks 2009). Finding the appropriate dosage for PPH management has proved particularly controversial due to the relative lack of efficacy of misoprostol compared with oxytocin (Gulmezoglu et al., 2001). This has led practitioners to use the maximum tolerated dose for PPH management.

The most commonly reported side effect of misoprostol is shivering, which is commonly followed by an increase in the body temperature. However, these side effects are not severe, are transient (resolving within 12 hours or less) and have not been associated with any lasting effect on health (Gulmezoglu et al., 2001; Hofmeyr et al., 2001b). The thermogenic activity of prostaglandin E was recognised in the human with the increasing use of high doses in reproductive medicine. Pharmacokinetic studies show that the sublingual and oral routes achieve higher and more rapid maximum plasma concentrations than the vaginal and rectal routes (Khan et al., 2004; Tang et al., 2002; Zieman et al., 1997). They would therefore be expected to be associated with a higher incidence of shivering and fever compared to the other routes.

The use of misoprostol for the prevention and treatment of PPH appears to be a particular problem with respect to the thermogenic side effects. In early studies of misoprostol a woman developed severe pyrexia with a rectal temperature of 41.9°C after ingestion of 800 mcg oral misoprostol (Chong, Chua & Arulkumaran 1997). A PPH treatment trial of 244 women also reported three women with a temperature above 40.0°C following 1000 mcg misoprostol (administered as 200 mcg oral, 400 mcg sublingual and 400 mcg rectal) (Hofmeyr et al., 2004). The most two recent

randomised controlled trials have confirmed that shivering and pyrexia are the most common side effects of 800 mcg sublingual misoprostol (Blum et al., 2010; Winikoff et al., 2010). Data from these trials showed that the incidence of shivering was 43%, with 34% reporting fever. In one hospital in Ecuador the incidence of high fever ≥ 40.0°C was 35.6% (Durocher et al., 2010). Fortunately, all cases made full recovery after management with cold compresses and antipyretics.

The effectiveness and safety of misoprostol are equally important for the clinician and for their patients. However, most of the studies which investigated misoprostol for the management of PPH have focused on the effectiveness of treatment and have paid little attention to the associated side effects. Although experience has shown that the misoprostol-related fever is not life-threatening, it would be better for patients' comfort, providers' acceptance and drug compliance if a lower effective dose could be found that minimises the rate of this side effect. In this chapter, we have therefore reviewed the incidence and risk of fever associated with the use of misoprostol for the prevention of PPH.

2. Objectives

To assess the incidence and risk of fever associated with the use of misoprostol for the prevention of PPH and examine how they vary with different doses and routes of administration.

3. Criteria for considering studies for this review

There is now a vast amount of literature on misoprostol use in the third stage of labour. Fever is, however, a very specific complication whose detection will be markedly increased if specifically sought in those using misoprostol, either through a questionnaire or through direct measurement. This is most likely to be done in the context of a randomised trial and we therefore focused our review on data from these studies only as a way of reducing reporting bias (Loke, Price & Herxheimer 2008; McIntosh, Woolacott & Bagnall 2004). Studies in which misoprostol was used for PPH treatment were excluded as haematological instability and blood loss could affect the thermoregulatory response of the body to misoprostol.

All misoprostol arms from randomised controlled trials were included in the analysis of the incidence of fever and grouped by route and dose. In the meta-analysis of fever risk, the ideal comparator would be a placebo. Given the evidence in favour of PPH prophylaxis, however, most researchers believe it to be unethical to use a no-treatment control. Placebo-controlled misoprostol trials for PPH prevention are therefore rare. In view of this, all the studies of misoprostol for PPH prevention have been retained for the risk meta-analysis, including studies that compare misoprostol with oxytocin, ergometrine and other misoprostol regimens. This is justified as fever is not a reported side effect of any of these therapies (Begley 1990; Prendiville et al., 1988).

2.1. Types of studies

All randomised clinical trials using misoprostol in at least one of their intervention arms for the prevention of PPH up to 20 July 2010.

2.2. Types of participants

The participants were women during the third stage of labour taking part in randomised trials in which prophylactic misoprostol was studied for the prevention of PPH.

2.3. Types of interventions

Misoprostol administered by any route or dose in one or more of the treatment arms and placebo or any other uterotonic in the control arm.

2.4. Types of outcome measures

The primary outcomes are the frequency of fever (authors' definitions) in the misoprostol group and its risk ratio compared to controls. Definitions of 'fever' vary considerably and so if the analysis were to be restricted to any particular cut-off, the number of trials to be included would be greatly reduced. It is therefore, considered justified to use 'fever' as an outcome and not a more specific cut-off.

4. Search strategy for identification of studies

In this narrowly focused review, the search in Cochrane CENTRAL, Scopus, Embase, Web of Knowledge and PubMed for randomised clinical trials was conducted using different free text terms including: misoprostol, third stage of labour (or labor), and postpartum haemorrhage (or hemorrhage). The search was limited to randomised clinical trials in humans without language restriction.

5. Methods for the randomised clinical trials

5.1. Studies selection

All potential studies identified as a result of the search strategy were assessed independently for inclusion by 2 reviewers (Anisa Elati & Andrew Weeks).

5.2. Data extraction and management

We developed a data extraction form based on the CONSORT statement for better reporting of harms in randomised trials (Ioannidis et al., 2004) (Appendix A.1) One reviewer extracted the data from the included studies and the second reviewer checked the extracted data. Discrepancies were resolved through discussion. StatsDirect Software and Review Manager 5 were used for data management, analysis and production of Forest plots.

5.3. Appraisal of study quality and data abstraction

We excluded non-randomised trials, those which used misoprostol for treatment of PPH, and trials which had not recorded the rate of fever. The validity of each remaining study was assessed by two reviewers working independently to determine the adequacy of randomisation and concealment of allocation, blinding and extent of loss to follow up according to the following criteria:

(1) Selection bias (randomisation and allocation concealment)
   (A) Adequate concealment of allocation: such as telephone randomisation, consecutively numbered sealed opaque envelopes;
   (B) Unclear whether adequate concealment of allocation: such as list or table

used, sealed envelopes, or study does not report any concealment approach;
(C) Inadequate concealment of allocation: such as open list of random number tables, use of case record numbers, dates of birth or days of the week.

(2) Attrition bias (loss of participants, e.g. withdrawals, dropouts, protocol deviations)
(A) less than 5% loss of participants;
(B) 5% to 9.9% of loss of participants;
(C) 10% to 19.9% loss of participants;
(D) More than 20% loss of participants.

(3) Performance bias (blinding of participants, researchers and outcome assessment)
(1) blinding of participants (yes/no/unclear);
(2) blinding of caregiver (yes/no/unclear);
(3) blinding of outcome assessment (yes/no/unclear).

5.4. Measures of treatment effect and assessment of heterogeneity

Tests of heterogeneity were applied between trials, using the I^2 statistic. In the absence of significant heterogeneity (exceeding 50%), fixed-effects meta-analysis was used for combining data. When there were high levels of heterogeneity, a random-effects meta-analysis was used to obtain the overall summary statistic. In this group, sensitivity analysis was also carried out to explore the effects of trial quality. This involved analysis based on the rating of selection and attrition bias. Studies of less than 'A' quality for these outcomes were excluded from the analysis in order to assess for any substantive difference to the overall result. The test for heterogeneity between the subgroups was used to explore the dosage effects. Heterogeneity was also explored by examination of year of publication. This is because postpartum fever is very prone to reporting bias, and it might be expected that reports of fever in early studies might lead to increased detection in later studies. Fever rates in the control groups were also explored as high reported fever rates might reflect population differences in peri-partum infection or in predisposition to fever, and may not occur simply as a result of the misoprostol.

7. Statistical analysis

Two main analyses were conducted. In the first, the incidence of fever was presented as the rate of fever in women who received misoprostol via different routes. Pre-specified subgroups were the doses of misoprostol. The data was summarised as an overall incidence for each route using StatsDirect software.

In the second analysis, the risk ratio of fever in studies comparing misoprostol to controls were combined in a formal meta-analysis and presented as risk ratios (RRs) with 95% confidence intervals (CIs) using Review Manager 5. In this second analysis, the following subgroup analyses were included:

I. The risk of fever with misoprostol versus placebo

1. 600 mcg oral misoprostol
2. 600 mcg sublingual misoprostol

II. The risk of fever with misoprostol versus oxytocin

1. 400 mcg oral misoprostol
2. 500 mcg oral misoprostol
3. 600 mcg oral misoprostol
3. 800 mcg oral misoprostol
4. 400 mcg sublingual misoprostol
5. 600 mcg sublingual misoprostol
6. 500 mcg rectal misoprostol
7. 600 mcg rectal misoprostol
8. 800 mcg rectal misoprostol

III. The risk of fever with misoprostol versus methyl ergometrine

1. 200 mcg oral misoprostol
2. 400 mcg oral misoprostol
3. 600 mcg oral misoprostol
4. 400 mcg sublingual misoprostol
5. 600 mcg sublingual misoprostol

IV. The risk of fever with misoprostol versus combined oxytocin/ergometrine

1. 400 mcg oral misoprostol.
2. 600 mcg oral misoprostol

V. The risk of fever with misoprostol compared to misoprostol of other doses and routes

1. 600 mcg versus 400 mcg sublingual
2. 600 mcg versus 400 mcg oral
3. 600 mcg versus 400 mcg rectal

8. Results

8.1. Description of the identified studies

For this review 231 studies were initially identified. After exclusion, 33 trials were included in the review and 28 were excluded (Figure 1).

8.1.1. Excluded studies

159 studies were excluded initially because the misoprostol was not being used for the management of the third stage of labour. A further 28 trials were excluded on closer examination: 2 trials were subgroup analyses for other trials included in the review, in 5 trials the misoprostol was used for treatment of PPH, 2 RCTs were excluded because misoprostol was mixed with other uterotonics in the treatment arm, one RCT has herb in the control arm (Miller 2009) and 19 trials did not provide data on the incidence of fever.

8.1.2. Included studies

We included 33 studies in the review, containing 38478 women. All of them were included in the assessment of the incidence of fever. We further excluded 3 RCTs in the meta-analysis of the risk of fever because the control group was a herb in 1 RCT and was mixed uterotonics in 2 RCTs.

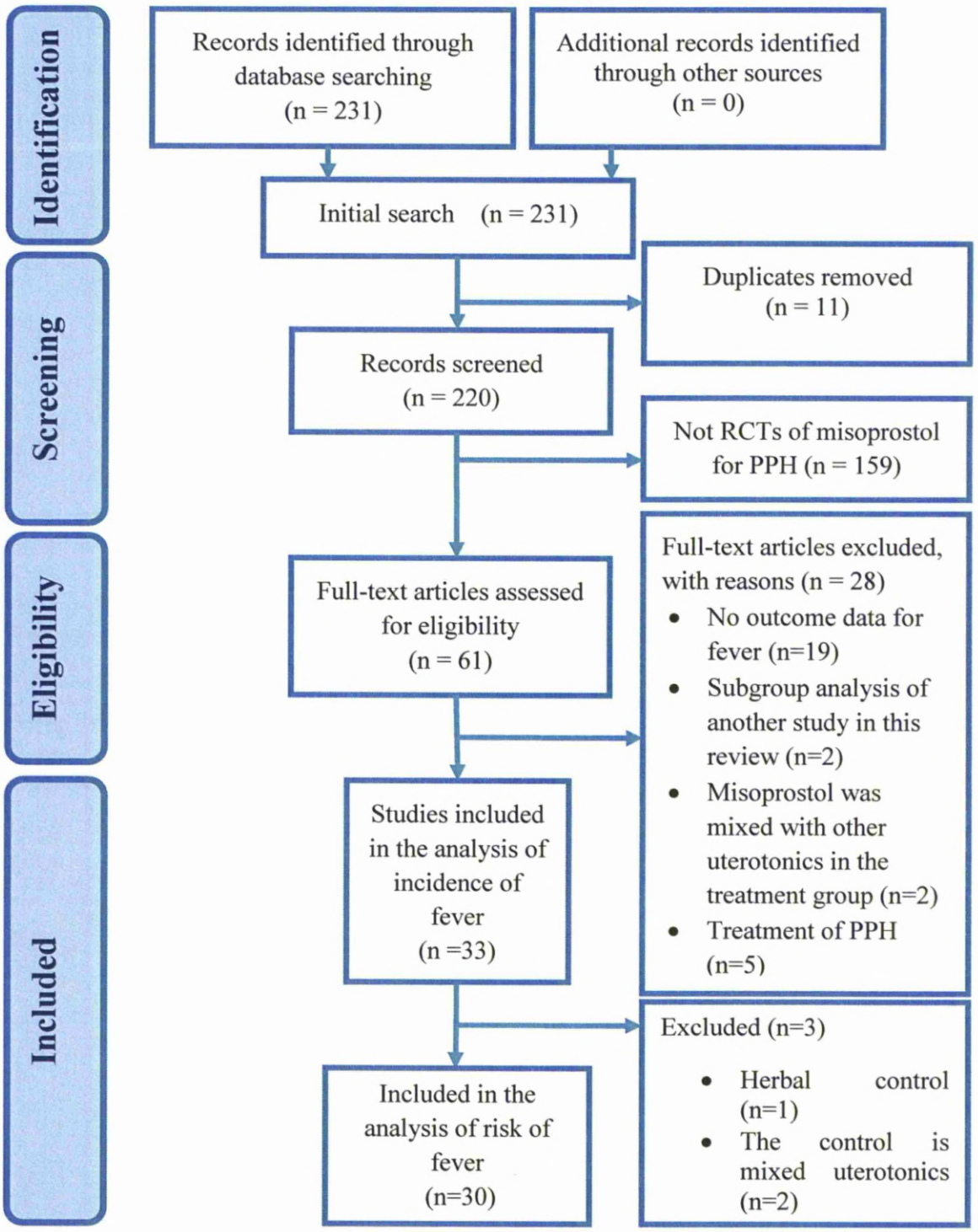

Figure 1. Flow diagram of the randomised clinical trials included in the review

8.2. Methodological quality of the included studies

8.2.1. Selection bias and attrition

All the included studies were randomised trials and 8 out of the 33 included studies were multicentre randomised controlled trials. 27 (out of 33) studies used adequate methods of allocation and concealment and 24 of the 33 reported loss of participants < 5%. For detailed description of the included trials see Appendix A.2.

8.2.2. Quality of reporting of fever

Five of the 33 trials had no clear definition of the side effect under investigation (Miller 2009; Patted 2009; Vimala 2006; Lokugamage 2001; El-Refaey 2000). The method of fever assessment was not clearly reported in 6 (Cook 1999; Garg 2005; Hofmeyr 2001; Miller 2009; Verma 2006; Naser 2009).

8.2.3. Performance bias (blinding)

Of the 33 included studies, only 19 trials were blinded. However, both blinded and open trials were included in the meta-analysis because the blinding was not possible in many good quality trials as they compared tablets and injection. This could be a source of bias if fever is not monitored systematically for all the participants, as the clinician who is aware of the fever as a common side effect of misoprostol may seek the complication more actively in women who had misoprostol.

8.3. Outcomes

8.3.1. Incidence of misoprostol induced fever

The incidence of fever was pooled according to the route of administration and the dose. There were 7 studies reporting fever with the sublingual misoprostol with a pooled incidence of 15% (95% CI 10-22, I^2 = 79.3%). The incidence was 12% (95% CI 7-18, I^2 = 58%) in the 400 mcg subgroup and 23% (95% CI 19-28) in the 600 mcg subgroup. The overall I^2 of 79.3% suggests a dosage effect (Figure 2).

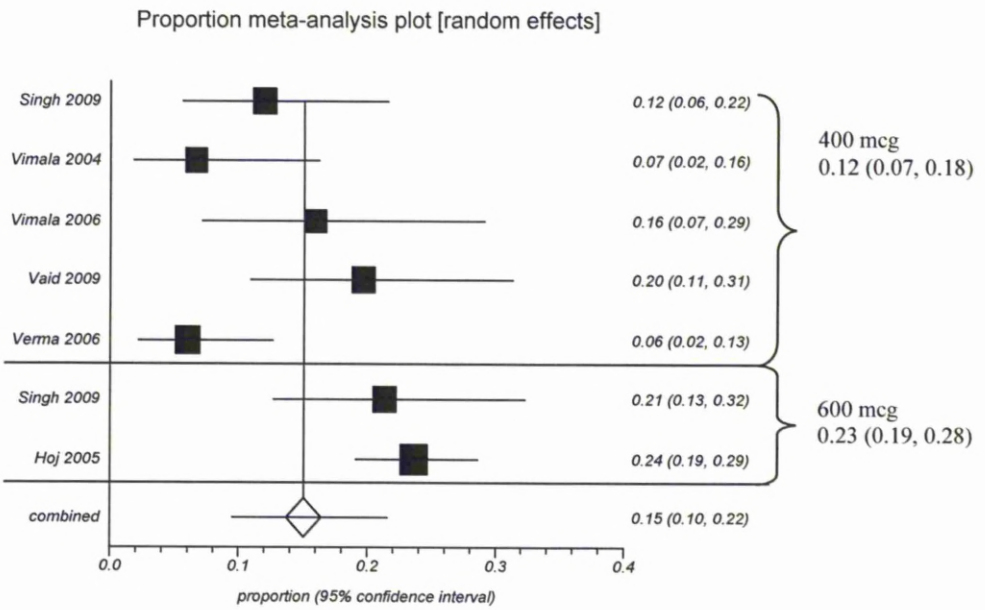

Figure 2. Pooled incidence of fever with sublingual misoprostol

The rate of fever with oral misoprostol was reported in 22 trials and the pooled incidence was 8.9% (95% CI 6.7,-11.4; $I^2 = 95\%$. The incidence was 6% (95% CI 2.2-12.6) in the 200 mcg subgroup (1 RCT), 7.5% (95% CI 5-10.5, $I^2 = 85.8\%$ in the 400 mcg subgroup and 10.6% (95% CI 6.6 -15.4, $I^2 = 97.2\%$ in the 600 mcg subgroup (Figure 3) and for the 800 mcg the incidence was 11.4 (95% CI 7.4-16.5) (1 RCT). The overall heterogeneity suggesting dosage effects between the subgroups.

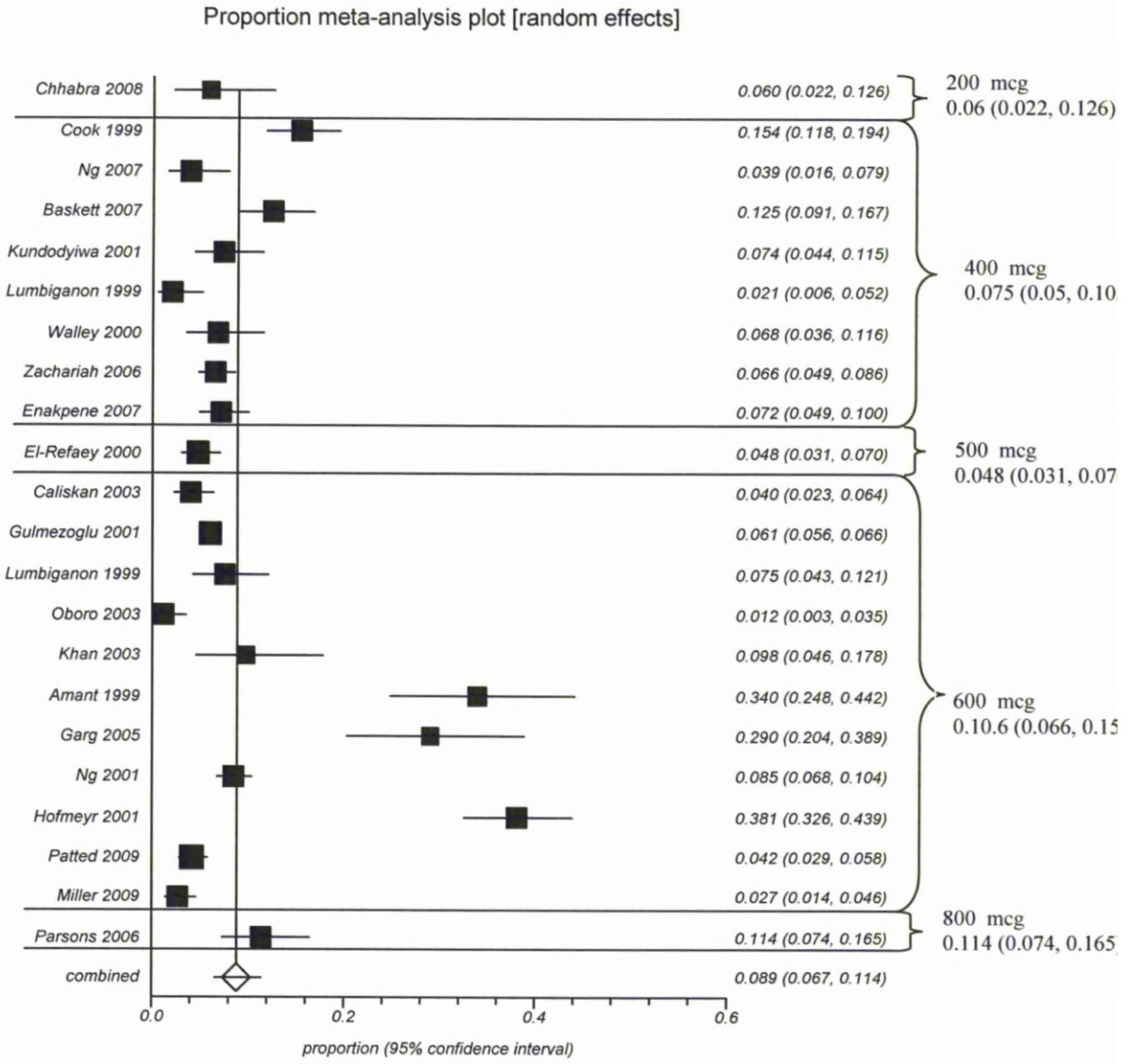

Figure 3. Pooled incidence of fever with oral misoprostol

The incidence of fever with rectal misoprostol was pooled from seven trials and gave a figure of 3.95% (95% CI 1.4-10.1; I^2 (inconsistency) = 91%. The incidence was 1.34% (95% CI 0.02-4.7) in the 400 mcg subgroup, 4.0% (95% CI 2.3-6.5) in the 500 mcg subgroup, 2.0% (95% CI 0.5-4.4) in the 600 mcg subgroup and 10.4% (95% CI 0.8-28.7) in the 800 mcg subgroup (Figure 4).

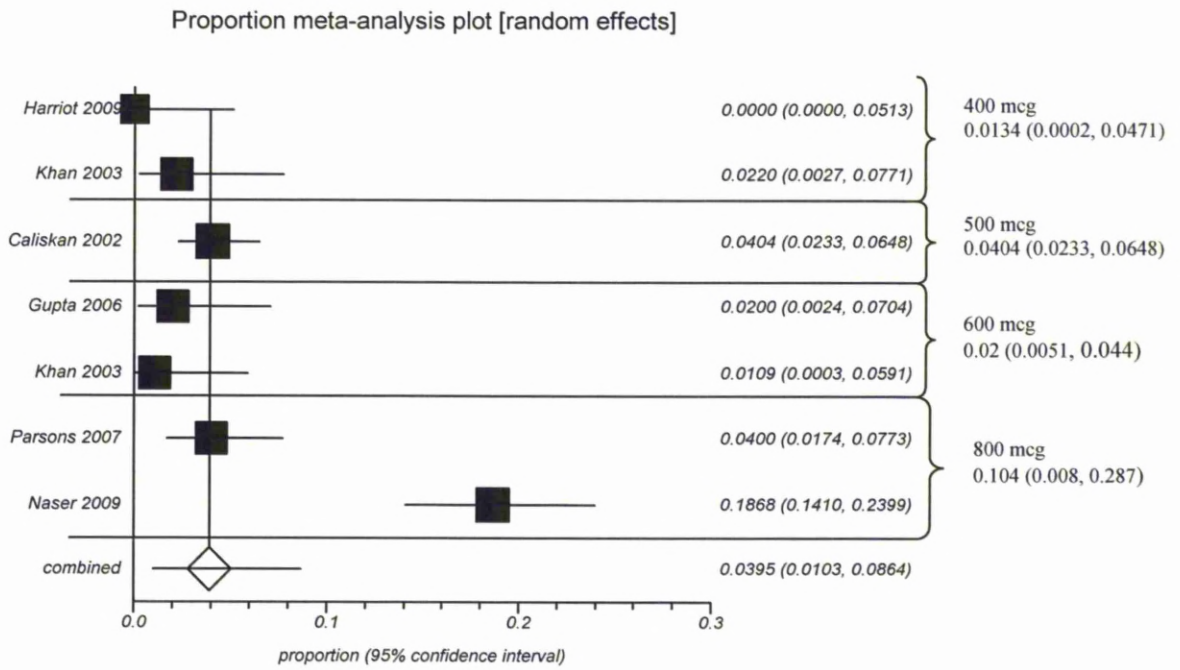

Figure 4. Pooled incidence of fever with rectal misoprostol

8.3.1.1. Exploring heterogeneity

The heterogeneity was explored through the dosage subgroups (see above). In addition further analyses were undertaken to explore the effect of date of publication (a potential increase in reporting the adverse drug reaction with time) and the ethnicity of participants.

8. 3.1.2. Year of study publication

Although there is a change in route of misoprostol used over time with sublingual misoprostol becoming more popular after 2004 there were no particular trends over time. The incidence of fever in Figure 5 is shown according to year and route.

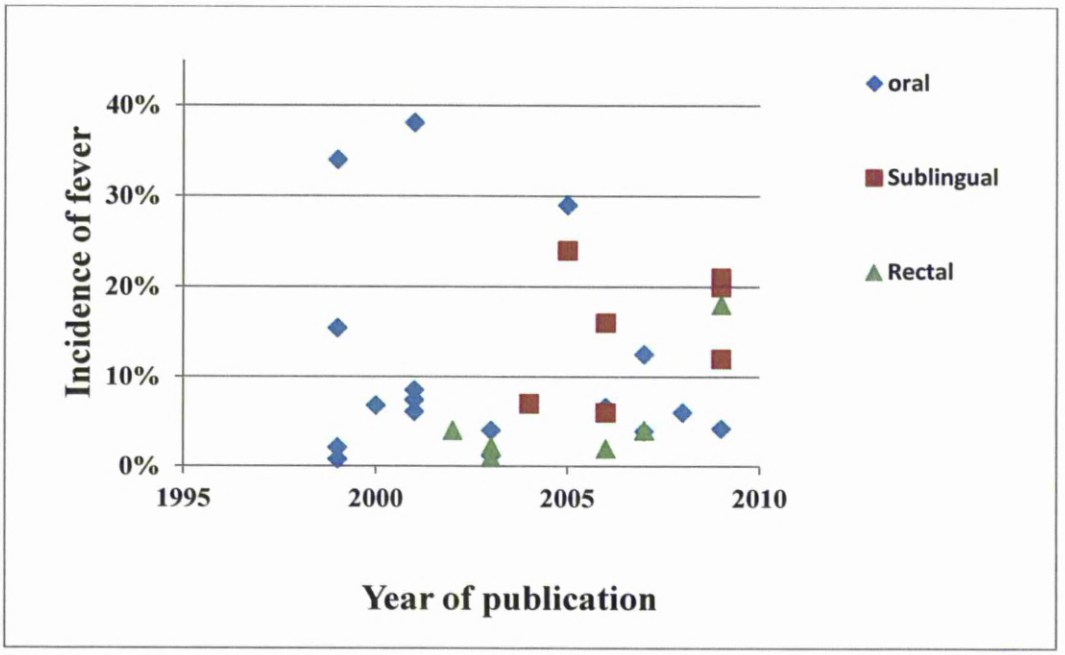

Figure 5. The incidence of fever with different routes of misoprostol according to the year of study publication

8.3.1.3. Ethnicity

The incidence of fever in single centre study (25 RCTs) was compared in the countries in which the study was conducted (breakdown of the incidence of fever according to participants' ethnicity was not provided in any of the studys' report). There were 2 studies in which the incidence of fever was far higher than the others: these were the studies using oral misoprostol conducted in Belgium and South Africa (Figure 6).

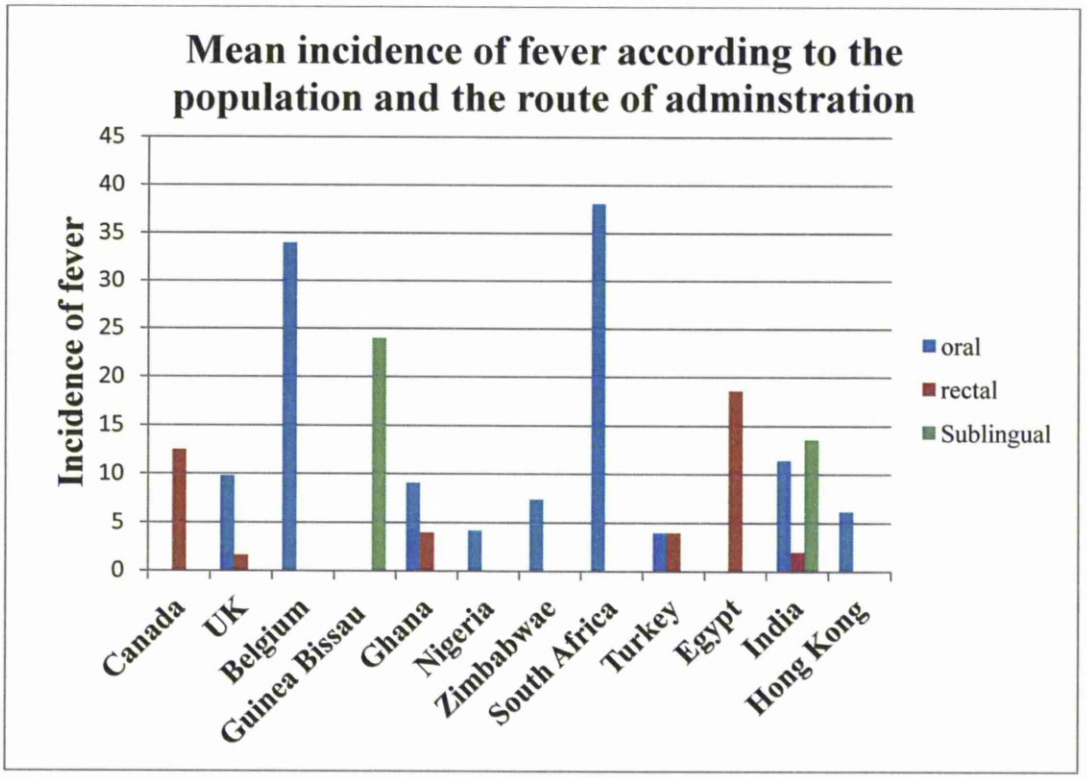

Figure 6. Incidence of fever with different routes of misoprostol according to the population

8.3.2. Risk of misoprostol induced fever compared to control groups

8.3.2.1. Risk of fever with misoprostol compared to that with placebo

There were only two studies comparing 600 mcg oral misoprostol with placebo which reported data on fever (Figure 7). The risk ratio (RR) of fever was 5.24 (95% CI 3.28-8.38). The risk of fever with 600 mcg sublingual misoprostol versus placebo was reported in only one study. The risk ratio was 7.11 (95% CI 3.85-13.12). The overall risk ratio of all 3 studies was 5.83 (95% CI 4.18-8.13). There was no overall heterogeneity or heterogeneity between these subgroups.

Of interest is the variation of the absolute risk between the studies. Although very homogenous results are found in the meta-analysis, the incidence of fever varies greatly between the groups with 38% in the Hofmeyr study, 24% in the Hoj study and 4.2% in the Patted study. The risk ratio is similar in the 3 studies however due to the large variation in the incidence of fever in the placebo groups (Hofmeyr 6.1%, Hoj 3.3% and Patted 1.1%).

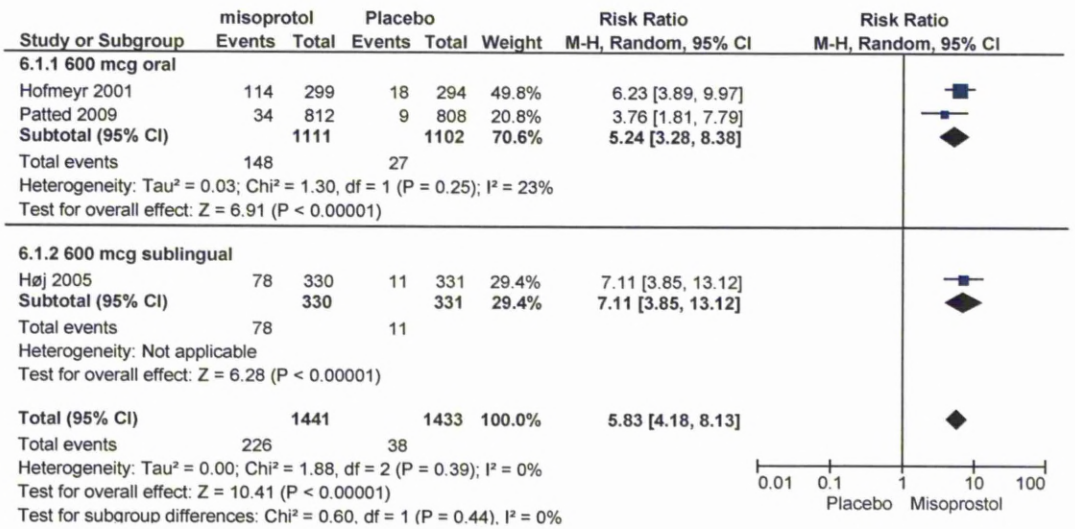

Figure 7. The risk ratio of fever with misoprostol versus Placebo

8.3.2.2. Risk of fever with misoprostol compared to that with oxytocin

Nineteen trials reported fever data in studies of misoprostol where oxytocin was the control (Figure 8). The risk ratio in the oral 400 mcg subgroup was 4.19 (95% CI 1.37-12.77, I^2 = 85%). Four studies reported the incidence of fever with 600 mcg oral misoprostol and the overall risk ratio was 4.22 (95% CI 2.12-8.38; I^2 = 60%). One study reported fever with 800 mcg oral misoprostol and this had a high relative risk (RR 50.62, CI 3.10-827.06).

For the sublingual route, there were 2 RCTs which reported fever as a side effect with 400 mcg sublingual misoprostol. The overall risk ratio was 5.67 (95% CI 1.48-21.71). The one study of 600 mcg sublingual misoprostol (Singh 2009) showed a risk ratio of 19 (95% CI 1.13-320.67).

Only one study reported data on fever with 500 mcg rectal misoprostol (RR 2.74, 95% CI 1.08-6.93) and one with 600 mcg rectal misoprostol (RR 5.00, 95% CI (0.24-102.8). Two studies reported data on fever with 800 mcg rectal misoprostol and gave a risk ratio of 6.9 (95% CI 0.52-91.94).

The overall risk ratio of fever with different doses and routes of misoprostol when compared to oxytocin was 4.94 (95% CI 2.77-8.8). There was a large amount of heterogeneity (78%) but the test for subgroup differences was I^2 = 0% (P=0.56) which shows no heterogeneity between the subgroups of doses and routes of misoprostol. All the heterogeneity was within the subgroups.

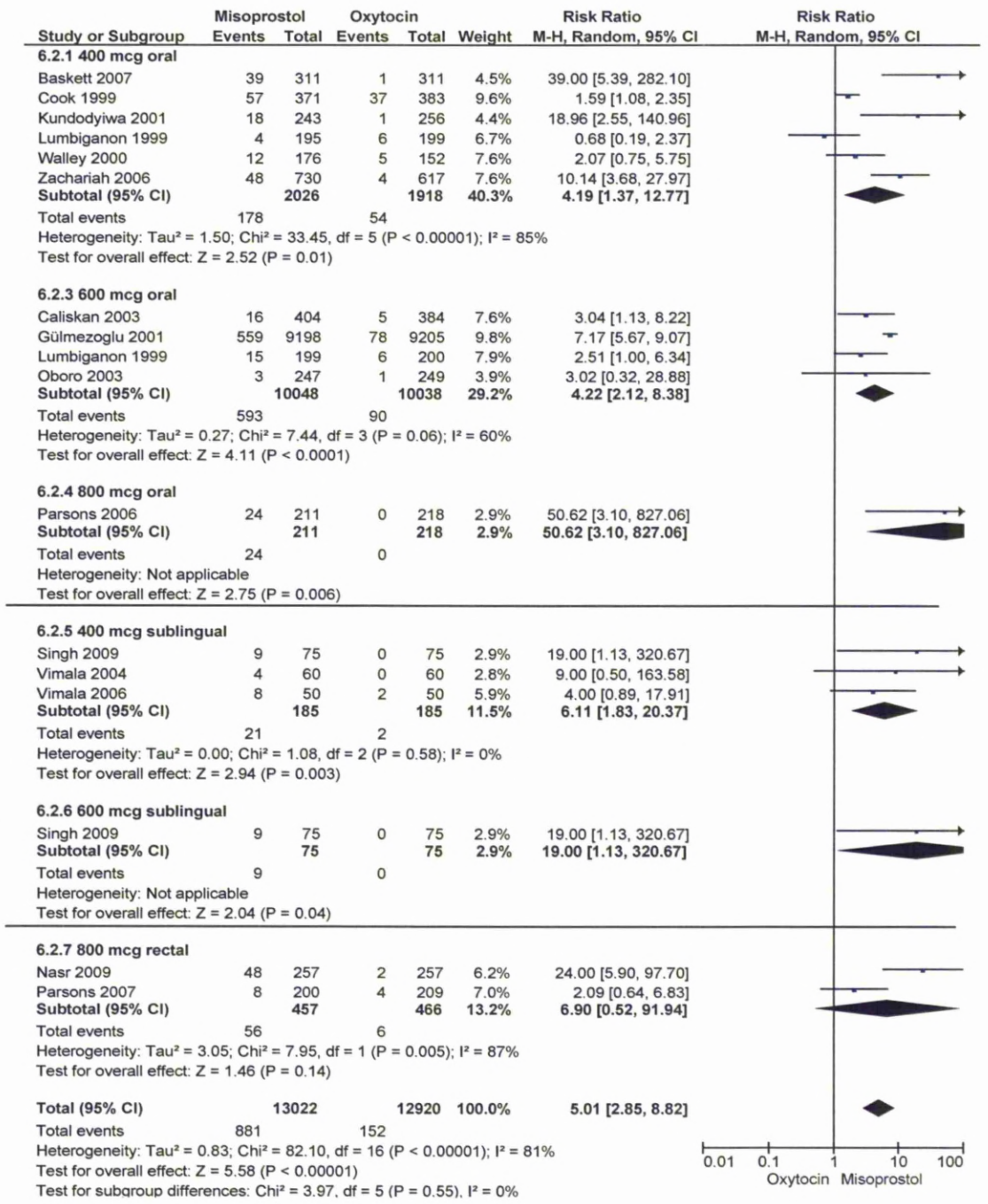

Figure 8. The risk ratio of fever with misoprostol versus oxytocin

8.3.2.3. Risk of fever with misoprostol compared to that with methyl ergometrine

The risk ratio of fever in the only study in which 200 mcg oral misoprostol was compared to methyl ergometrine was 13 (95% CI 0.74-227.72) (Figure 9). Two studies reported data on fever with 400 mcg oral misoprostol and the risk ratio was 3.75 (95% CI 2.31-6.09). Also, two studies showed data on fever with 600 mcg oral misoprostol (RR 6.28, 95% CI 2.32-16.96).

In considering the risk of fever for the sublingual route versus methyl ergometrine, 4 studies reported the risk of fever with 400 mcg sublingual misoprostol. The risk ratio was 10.29 (95% CI 3.17-33.46). Only one study gave data on fever with 600 mcg sublingual misoprostol (RR 33, 95% CI 2.02-540.22).

The overall risk ratio of fever with different doses and route of misoprostol versus methyl ergometrine was 4.97 (3.47-7.14). The overall heterogeneity was 0% and the test for subgroup differences (I^2) was about 22.6% with p value of 0.27, which suggested no heterogeneity within or between the subgroups.

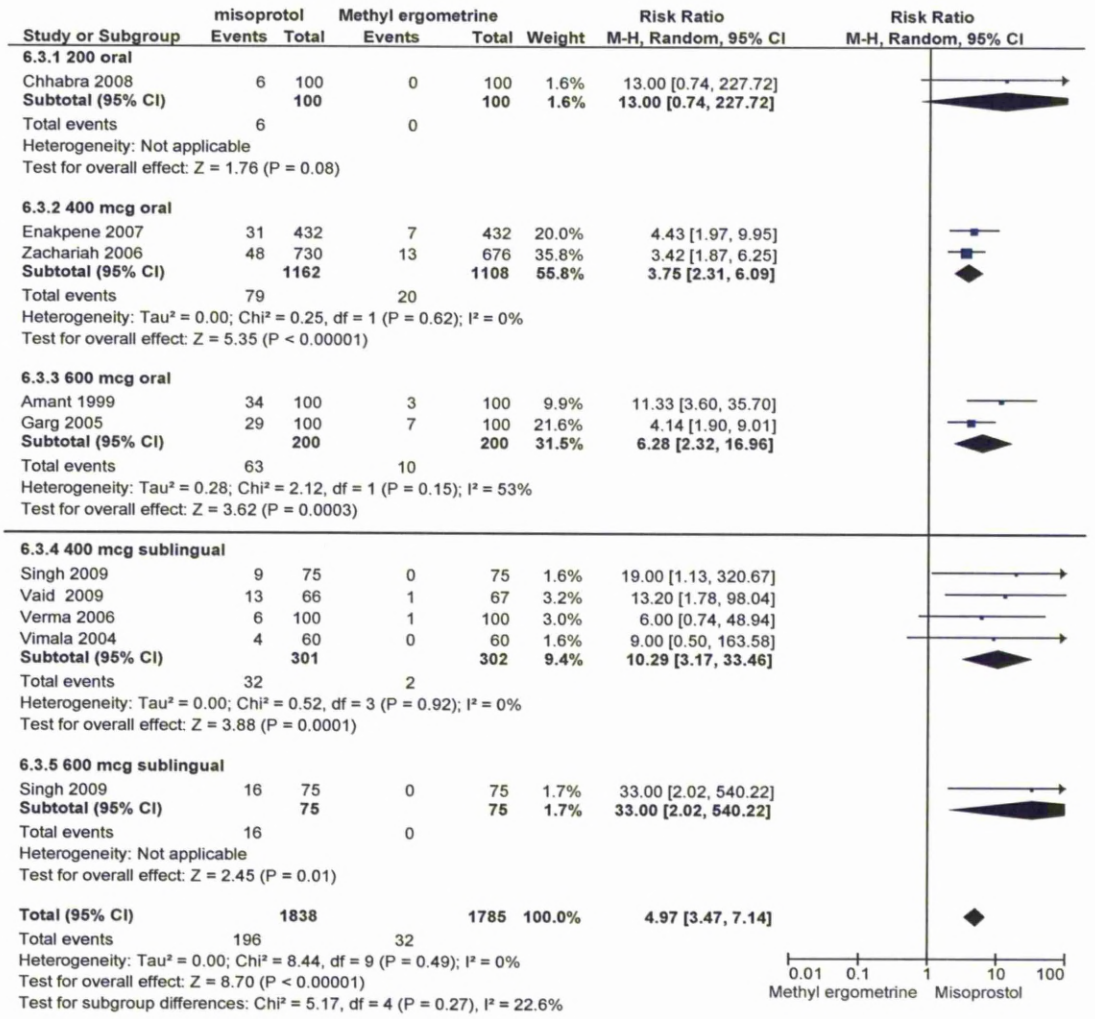

Figure 9. The risk ratio of fever with misoprostol versus methyl ergometrine

8.3.2.4. Risk of fever with misoprostol compared to that with syntometrine®

There were two studies reported data on fever with 400 mcg oral misoprostol and the risk ratio was 3.13 (95% CI 0.4-24.66) (Figure 10). The risk ratio with 600 mcg sublingual misoprostol was 6.73 (95% CI 3.78-11.98). The overall risk for misoprostol in studies in which it was compared with syntometrine was 4.04 (95% CI 1.06-15.48). The test for subgroup differences (I^2) was 0%, (P=0.04) which suggested no dosage effect.

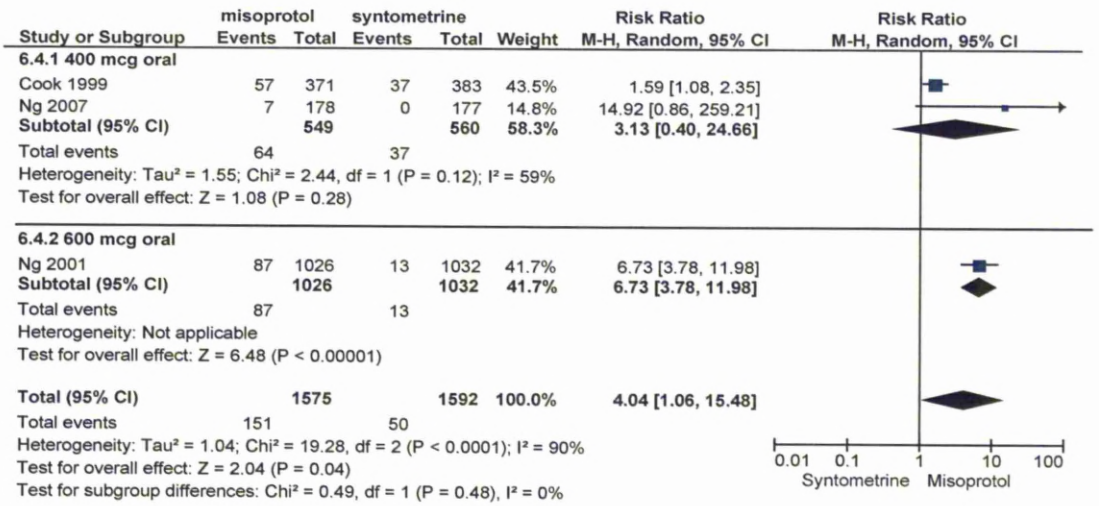

Figure 10. Risk of fever with different doses and routes of misoprostol versus syntometrine for prevention of PPH

8.3.2.5. Risk of fever with misoprostol compared to that with misoprostol of other doses or routes

Three studies compared high to low doses of misoprostol (Figure 11). Singh 2009 compared 600 mcg and 400 mcg sublingual misoprostol (RR 1.78, 95% CI 0.84-3.77). Lumbiganon 1999 compared 600 mcg to 400 mcg oral misoprostol (RR 3.73, 95% CI 1.26-11.04) and Khan compared 600 mcg and 400 mcg rectal misoprostol (RR 0.49, 95% CI 0.05-5.36). The overall risk ratio favours the lower doses with RR 2.13, 95% CI 1.19-3.81).

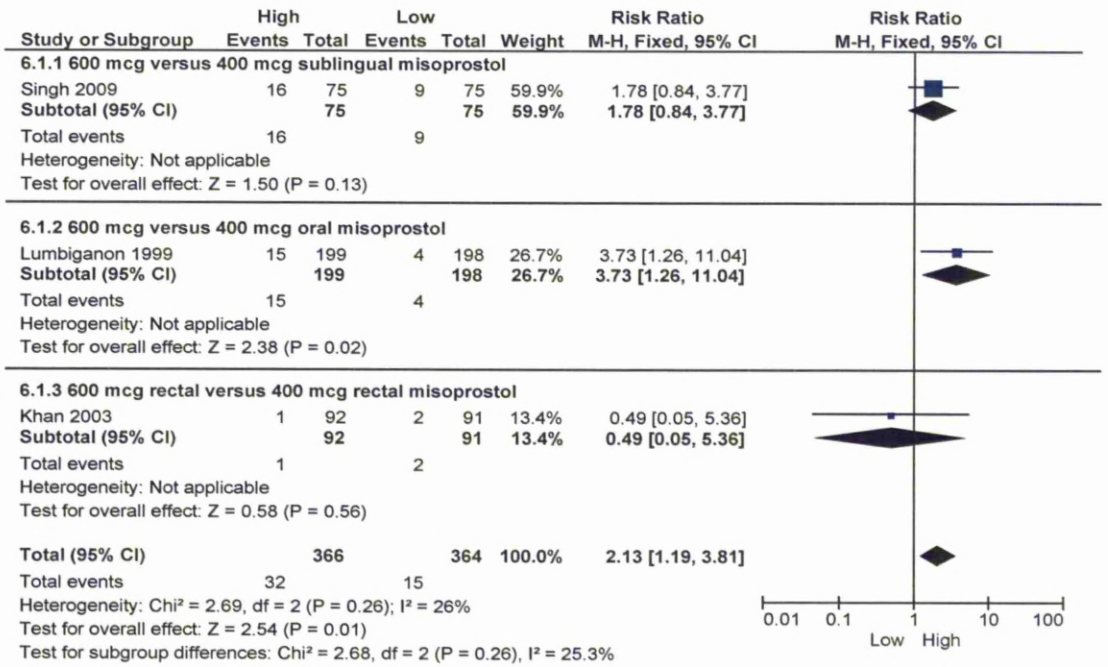

Figure 11. Risk of fever with misoprostol compared to that with misoprostol of other doses or routes

9. Discussion

The review has shown that the incidence of misoprostol induced fever was route related as the incidence with sublingual misoprostol was higher than oral and the latter was higher than the rectal. Also, the frequency of fever was dose related. The overall risk of fever was 5 times higher with misoprostol compared to placebo or any other uterotonics.

The overall incidence of fever by different routes of misoprostol showed the highest incidence with sublingual route (15%) followed by the oral route (11.4%) and the rectal route rate (4%). This might be explained by the pharmacokinetics and the pharmacodynamics properties of different routes of misoprostol. The difference in the rate of fever with different doses and routes of misoprostol is also consistent with the direct comparison of misoprostol in this review. The comparison was between 600 mcg oral, 400 mcg rectal and 600 mcg rectal misoprostol and the incidence of fever was 9%, 2% and 1% respectively (Khan & El-Refaey 2003). Three recent studies that were published too late for inclusion in this review. One pharmacodynamic study showed that the rates of fever over 39°C in sublingual doses of 200, 400 and 600 mcg were 8.3%, 8.3% and 45.4% (Elati et al., 2011). The other two RCTs also showed low incidence of fever >38 °C with 400 mcg sublingual misoprostol which was 6% (Fawole et al., 2011) and 2.3% (Chaudhuri, Biswas & Mandal 2012).This data all supports the notion that there is both a dosage and route effect on the incidence of fever with misoprostol. Sublingual routes achieve a higher peak concentration compared to the other routes of administration as a result of its rapid absorption through the sublingual mucosa and the avoidance of the first pass metabolism via the liver (Tang, Gemzell-Danielsson & Ho 2007).

Interestingly however, there is no clear dosage or route effect seen in the studies of risk ratio. This fact points to wide variation in the incidence of fever in the control arms of the studies as well as the misoprostol arm. This is best demonstrated in the placebo controlled studies where the wide variation in incidence in the misoprostol arms of the 3 studies are cancelled out by equally wide variations in the incidence in the placebo arm leading to a homogenous finding in which the risk ratio was around 5 in all the studies. The reason for the wide variation in postnatal fever in the reported

studies is not known but may be occur as a result of clinical factors such as pre-partum infection, multiple vaginal examinations with prolonged labour and prolonged rupture of membranes. Reported rates of fever may also vary according to whether the temperature is systematically and regularly measured post-delivery, self-reported, or simply retrospectively collected from case notes after the event. Given that in many settings temperature is not routinely measured in the immediate postnatal period and midwives may not formally record the symptom if it settles spontaneously, the latter methods may grossly underestimate the incidence of this side effect. To provide accurate data about the risk of fever, we recommend systematic collection and reporting of postnatal temperature in future randomised controlled trials.

An alternative explanation for the different rates of fever between studies could be a genetic variation in participants' predisposition to side effects. Apost hoc analysis of a clinical trials for treatment of postpartum haemorrhage using 800 mcg sublingual misoprostol showed that the incidence of fever ($\geq$ 40.0 °C) was 35.6% in Ecuador compared to women from other populations who has lower incidence of fever.It was less than 3% in Turkey and Egypt (Durocher et al., 2010). Genotyping of DNA from women who have misoprostol induced fever may help the identification of variant genotypes with the highest predictive value for the development of chills and/ or fever in women treated with misoprostol. Misoprostol has a similar mechanism of fever production as PGE_2, which is a key fever mediator in the brain and ultimately in charge of the upward shift of the thermoregulatory set-point. Several genes are involved in the mechanism of action of prostaglandins including genes involved in the expression of prostaglandins receptors (PTGER1,2,3,4) and prostaglandin transporters (SLCO2A1, SLCO1B1 & ABCC4) in the brain (Oka, Oka & Saper 2003). There are also other genes which are involved in the expression of separate receptors that mediate the prostaglandin action such as gamma-aminobutyric acid receptor genes (GABRG2 & GABRA2; (Osaka 2008a) and adrenergic beta receptors genes (ADRB1,2,3 & ADRBK1; (Amir & Schiavetto 1990). Genetic polymorphisms in these genes may affect the response to misoprostol and the susceptibility of women to misoprostol-induced fever. Genetic studies were conducted to explore this possibility and this will be in shown in detail in Chapter 4.

Systematic reviews of adverse effects can be broad or narrow in scope. Broad scope reviews examine all the adverse effects associated with a particular therapy while a narrowly focused review examines a known or common side effect in detail (Loke, Price & Herxheimer 2007). This review is of the latter variety and examines the incidence and risk of fever as a commonly observed side effect of misoprostol. McIntosh and her colleagues studied the assessment of harmful effects in systematic reviews and concluded that systematic reviews of adverse effects were more likely to produce appropriate information if they addressed a focused question. They also suggested developing quality assessment methods for the trials of harmful effects to be used in these reviews (McIntosh, Woolacott & Bagnall 2004).

The decision on types of studies to include in the review depends mainly on the focus of the research question. Data on rare or new side effects of an intervention are unlikely to be found in reports from RCTs and may require the examination of all studies from a wide selection of studies types such as cohort, case-control, observational, randomised control trials and case series reports. In contrast, data on well recognised and easily detectable side effects may be available from RCTs (Loke, Price & Herxheimer 2007). This narrowly focused review evaluates fever which is common side effect of misoprostol. We therefore included only randomised control trials.

The reporting of harms in RCTs is often inadequate and receives less attention than the reporting of efficacy and effectiveness. In this review we had to exclude 19 trials because of lack of information about fever. Ideally the assessment of quality of trials to be included in the review of adverse effects should concentrate on how precise the methods were used to detect fever and how good the reporting was (Ioannidis et al., 2004). We assume that the method for examination of fever is by using the thermometer as a measurement tool. However, many studies might not mention this assuming it is already known practice for the measurement of fever and others may rely on participants' self reports. This is unclear in many study reports and may have been a contributing factor to the inconsistency in rates.

Inconsistency in terminology can also lead to difficulties when searching for studies for systematic reviews and lead to the missing of some important trials. Therefore, in

our search terms we did not include fever or hyperthermia as search terms, but instead searched for all the trials that using misoprostol for postpartum haemorrhage and then examined all of them to find reports of fever as a side effect.

10. Conclusion

The incidence of fever with misoprostol is related to both its dosage and route with the highest incidences found in the high dose sublingual route. However, this is not the only influence on postnatal fever. There appear also to be effects related to genetic variation between ethnic groups and intra-partum clinical factors. Further research is required to find out the low and best effective dose for prevention of PPH with the best adverse drug reaction profile.

Chapter 3

The effect of misoprostol on postpartum contractions: a randomised comparison of three sublingual doses

1. Introduction

PPH is the leading cause of maternal mortality in the developing world and is responsible for about 25% of all maternal deaths worldwide (Khan et al., 2006). The most common cause of PPH is the failure of the uterus to contract after child birth (atonic PPH) and AMTSL is recommended to prevent it (NICE 2007; WHO 2007). This involves, as a minimum, the administration of a uterotonic drug at delivery and controlled cord traction. Oxytocin is recommended as the first line oxytocic for the prevention of PPH (FIGO/ICM 2004a; WHO 2007). However, oxytocin requires refrigeration because it is unstable when exposed to high ambient temperatures. Furthermore, this drug must be given parenterally which requires a skilled birth attendant and a continuous supply of sterile syringes and needles. Both of these are frequently unavailable in low resource settings. About 99% of maternal deaths occur in low resource settings where there are poor transportation systems, a lack of skilled birth attendance and emergency obstetrics services (McCormick et al., 2002). Hence, a major objective for reducing maternal deaths in poor areas is to find low-cost and effective ways to prevent and control PPH.

Misoprostol is orally active prostaglandin analogue with uterotonic effects, and is an option for PPH prevention in low resource settings due to its thermo-stability, cost effectiveness and ease of administration. There have been at least 36 trials that have studied misoprostol for PPH prevention using doses of between 200 and 1000 mcg and a variety of routes including oral, vaginal, sublingual and rectal (Gulmezoglu et al., 2007). It is, however, as yet unclear which gives the best balance of efficacy and safety. Given the relative ineffectiveness of misoprostol for PPH, the tendency has been to use high doses of misoprostol. There are however potential dangers to this. Shivering and pyrexia are commonly reported side effects, and hyperpyrexia of over 40°C has been reported, reaching an incidence of 36% in some population groups (Winikoff et al., 2010). A recent systematic review recommends further research to find out the optimal route and minimum effective dose of misoprostol for routine use for the prevention of PPH (Hofmeyr et al., 2009). The authors suggest that the sublingual route is likely to be the most suitable owing to its rapid uptake, prolonged duration of action and greater bioavailability.

Early studies showed that medications such as oxytocin, ergometrine and prostaglandins all have a strong contractile effect on uterine muscle. Intrauterine pressure catheters were widely utilized to investigate the effect of these medications and to compare different doses and routes for prevention and treatment of PPH (Chong et al., 2001; Chong et al., 2004).

Intrauterine pressure (IUP) measurements were mainly introduced to clinical practice in order to study uterine activity during labour. They assisted in the diagnosis of labour dysfunctions such as obstructed labour, arrested labour and uterine hyperstimulation, particularly during induction of labour using uterotonics. The intrauterine pressure measurements were used extensively in research to investigate uterine activity at different stages of the female reproductive life. For instance, intrauterine pressure catheters were used in studies of uterine muscle activity during menstruation to investigate dysmenorrhoea (Lumsden, Kelly & Baird 1983; Milsom & Andersch 1985), and uterine stimulation during different procedures in various stages of assisted reproduction (Bulletti & De Ziegler 2005). They were also used in research into uterine activity during the third stage of labour to explore the causes and treatment of uterine atony (Chong et al., 2001).

The current methods of intrauterine pressure measurement came after more than a century of evolving and improving techniques for this purpose. There are many types of intrauterine pressure catheters (IUPC) (Figur 1). However, the most common types are the external transducer catheters and the tip transducer catheters. Catheters with a tip sensor but external transducer (e.g Koala) show some advantages over the catheter tip transducer (e.g Intran). It has a softer, smaller catheter tip and flexible body and is therefore easier to insert. Also, the accuracy of the measurements are not be affected by changes in the temperature from body to room temperature. It is also easier to set up and easier to zero while it is *in-utero* as the transducer is connected to an external reusable cable (Dowdle 1997). The tip sensors are of various types. There is a pinpoint tip sensor with a membrane sensitive to the surrounding pressure produced by a column of the amniotic fluid. The other type of tip sensors is a sensing membrane, or balloon on the tip of the catheter. It employs the air-coupling technology from a distally mounted flexible balloon (sensing membrane) that

communicates through a sealed micro-tube to the externally located electronic reusable transducer in the monitor cable and connector (Figure 1). This type of catheter was produced by Clinical Innovations and is known as Koala IUP-5000E catheter.

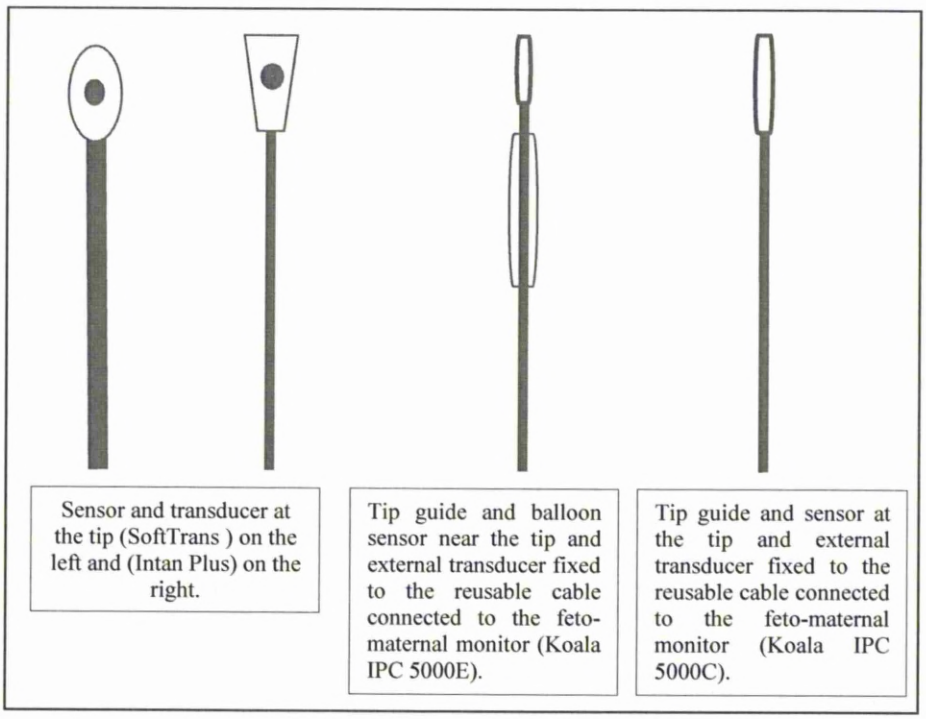

Figure 1. Types of the catheters according to the position of the sensors and the transducers

When the temperature sensitive transducer is placed at the tip of the catheter, the change from room to body temperature affects the monitoring baseline. The thermal effects combined with other sensitivities can alter the intrauterine reading by 8 mmHg. The presence of the transducer outside the uterus provides accurate re-zeroing just by disconnecting the catheter from the cable transducer and pressing the zero button on the fetal monitor. A true zero can be only obtained when the transducer is exposed to the atmospheric pressure (Anonymous 2009). To our knowledge there has been no studies that has used the external transducer catheter (Koala) to evaluate the treatment effect of uterotonics during the third stage of labour.

The reliability of the air-charged coupled Koala catheter has been compared with an electronic pressure transducer tipped catheter (Intran) during the first stage of labour

(Dowdle 1997; Dowdle 2003). Both catheters were bound together at their tips and introduced into the uterus (Dowdle 2003). The data showed a similar mean baseline tone, peak pressures, contractions frequency and duration during labour. However, neither reliability nor validity studies have been performed for the Koala catheter during the third stage of labour.

This chapter describes an in vitro study to examine the validity of an IUPC with an external transducer (Koala External IPC 5000E) and a catheter tipped transducer (Intran pluse IUP-500). This will enable an appropriate choice of catheter in the clinical trial to examine the uterine activity during the third stage of labour after administering different doses of sublingual misoprostol to prevent PPH.

2. Objectives and hypothesis

This study therefore was devised

1. To examine and compare the validity of the pressure values that given by Koala and Intran plus Catheters to find the best to be used in the clinical trial of comparing the IUP after three sublingual doses of misoprostol.

2. To compare the effects of three sublingual misoprostol doses using the measurements of postpartum intrauterine pressure as a surrogate endpoint to evaluate the uterine activity of these uterotonics

3. To compare the side effects associated with each treatment.

Data from a small cohort of women given intramuscular oxytocin prophylaxis are also shown for comparison with the misoprostol data.

We hypothesised that low doses of misoprostol produce a similar strength of myometrial contraction to high doses.

3. Materials and methods

3.1. The validity of the intrauterine pressure catheters

Two types of intrauterine pressure catheters were investigated. They are the Intran plus IUP-500 (Utah Medical Products, Utah, Inc, USA) which has a silicone diaphragm transducer on its tip, and the Koala External IPC 5000E (Clinical Innovations, Utah, USA) which has a sensing membrane near its tip. This communicates through a sealed micro-tube to an electronic transducer located in the reusable catheters (Figure 2). The procedure was carried out by two observers using three different methods.

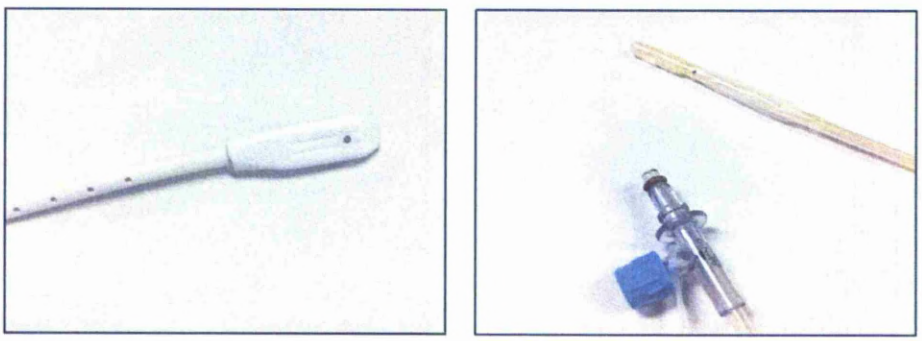

Figure 2. Intran plus IUP-500 (left) and Koala External IPC 5000E (right)

1. Testing accuracy in air with varying air pressures:

We measured the pressure inside a calibrator (Latitude® calibrator, Clinical Innovations, Utah, USA) (Figure 3). This represents the ideal situation for the intra uterine catheter to measure the pressure correctly. Both the Intran plus and the Koala External catheters were inserted into the calibrator until both sensors were inside and sealed into the end of tube. Each was connected to a feto-maternal monitor (General Electric, GE 250 series CX). The first observer pumped the tube with series of predetermined pressures between 0 and 120 mmHg (the maximum pressure in the calibrator pressure gauge). The second observer remained blind to the tube pressure but recorded the values of the pressure from the electronic tocograph on the two feto-maternal machines. The pressure values were selected at random within each range using a random number generator programme on (http://www.random.org) and then arranged in a random order using the same software. The pressure was selected in three categories (0-40, 41-80 and 81-120 mmHg). This ensured that the two catheters were tested at various levels of pressures and included multiple measurements. A total of 40 different measurements for each catheter were made available for the final analysis.

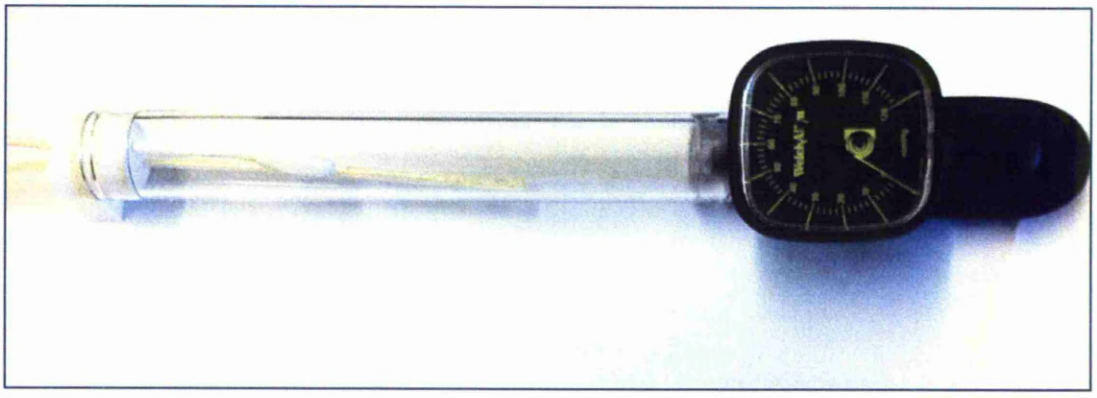

Figure 3. Latitude® calibrator with the two catheters inside

2. Testing accuracy in water with varying fluid pressures:

The second method attempted to mimic the situation in a fluid filled cavity. A rubber balloon filled with 5 ml of water was fixed around each catheter tip (Figure 4) and surrounded by a pressure cuff. Each catheter was connected to a feto-maternal monitor (General Electric, GE 250 series CX). Approximately 5 mls of tap water was put into the balloon around the catheters and a sphygmomanometer cuff placed around the balloon. The pressure exerted by the sphygmomanometer should be transmitted through the fluid to the catheters and then to the feto-maternal monitors. The pressure values measured by the monitors were compared to the actual pressure exerted by the sphygmomanometer (Gold standard). The first observer pumped the cuff to a predetermined pressure between 0 and 200 mmHg and shielded the sphygmomanometer dial from the first observer. The pressure values were selected at random within each range using a random number generator programme on (http://www.random.org) and then arranged in a random order using the same software. The pressure was tested in four categories (0-50, 51-100, 101-150 and 151-200 mmHg). This ensured that the two catheters were tested at various levels of pressures and included multiple measurements. A total of 80 different measurements for each catheter were made available for the final analysis.

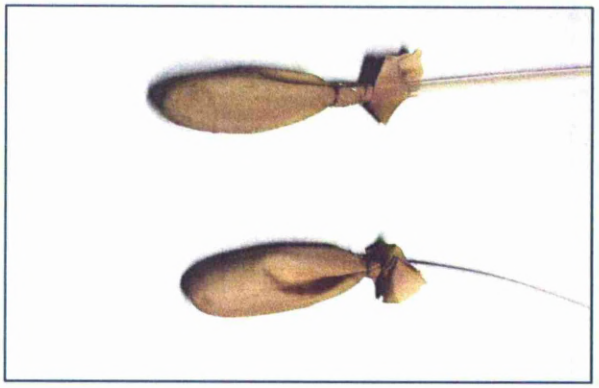

Figure 4. Intran pluse IUP-500 (below) and Koala External IPC 5000E (above), each within balloon filled with 5 ml of water

3. Testing accuracy in simulated (wet) postpartum uterine activity:

The validity of the two catheters was tested in an open but wet system designed to mimic the postpartum uterus using wet tissue surrounding the catheters' tips. Two catheters were fixed 1 cm apart using adhesive tape. The two catheters' tips were wrapped with wet tissue and which was moistened after each measurement (as water was squeezed out of the tissue each time the pressure was applied). This is similar to the postpartum uterus where there is a little amount of fluid (blood) that is squeezed out and yet produced again between each contraction (Figure 5).

Figure 5. Intran pluse IUP-500 and Koala External IPC 5000E, within wet tissue

To avoid bias, the second observer, who read and recorded the measurements from the two machines, was blind to the true measurements. The data was collected on two identical forms shown below. The first observer filled the column of true pressure values on one copy while the second observer filled the columns for the pressure values on measured by the two feto-maternal monitors on a second copy. At the end of the experiment, the two data forms were combined onto one form.

	Sphygmomanometer/ Calibrator (True)	**Koala**	**Intran**
1			
2			
3			
4			
5			
....80			

3.2. Randomised clinical trial

3.2.1. The study population and location

The study population was made up of women who gave birth at Zliten Teaching Hospital (in Zliten, Libya) between July and December 2009. This is a government funded, secondary referral hospital serving a population of 200,000 people with 4,500 deliveries per year. Prior to the study, a small pilot study was conducted in a nearby teaching hospital (in Misurata) in order to test and improve the study equipment and methodology which was employed for data acquisition.

Inclusion criteria

- Age more than 18 years old
- Women who have had spontaneous vaginal delivery
- Women with gestational age >34 weeks
- Women who give an informed written consent

Exclusion criteria

- Women with history of PPH
- Women with history of APH
- Women with previous caesarean section
- Women with 5 or more previous deliveries at over 28 weeks
- Women who have PROM
- Women with anaemia (Hb <10g/dl)
- Women who have given birth to a baby of birth weight > 4.0 Kgs
- Women who have induced or augmented labour with any uterotonic drugs
- Women with multiple pregnancies
- Women with polyhydramnios
- Women with pre eclampsia
- Women with infection
- Women who do not give a written consent

3.2.2. The study procedures

3.2.2.1. The pilot study

To my knowledge, our research methods, units of measurement and the summary measures (MVU) have not been used in previous studies looking at the intrauterine pressure postpartum as a parameter. Therefore, a pilot study was conduct before the original study in order to give more information about the nature and the characteristics of the postpartum intrauterine pressure. Also, the pilot study was conducted to explore the practicalities of the catheters, equipment setup and the data acquisition. The findings of this pilot study used to calculate the sample size for the final study.

10 participants were recruited according to the above mentioned inclusion and exclusion criteria. Intrauterine pressure catheter was inserted through the cervix immediately after the delivery of the placenta and held in place using the supplied adhesive pad against the mother's thigh. Initially, up to 5 women received 10 units of intramuscular oxytocin and the equipment and study instruments tested. Once consistent readings are being obtained, a further 5 participants received 600 mcg moistened sublingual misoprostol. The intrauterine pressure was continuously recorded over 120 minutes. Vital signs, pulse, blood pressure and temperature were measured at before treatment and at 30, 60, 90 and 120 minutes.

At the end of this small pilot study, the study was transferred to Zliten Teaching Hospital and the ethical approval was amended accordingly. During the trial set up period for the main study at this new site (pilot data analysis, recalculation of samples sizes and development of the randomisation schedule and envelopes), 14 women were recruited to an observational study at Zliten where women were treated with oxytocin alone. Once all the study instruments were in place, the main randomised study commenced and women entering the delivery suite at the times when the researcher was present (usually working hours during the week) were invited to participate.

3.2.2.2. Randomisation and concealment

A commercial randomisation programme was used to produce a random list of allocations to 3 doses of misoprostol (www.sealedenvelope.com). The allocations were written on cards and placed in consecutively numbered sealed opaque envelopes by staff not involved in the study. Blinding was not possible since the treatment was provided by the researcher who was available at the time of the delivery to carry out the final selection of women and collection of data.

3.2.2.3. The main study procedure

Women who met the inclusion criteria were invited to participate in the randomised study. After reading the patient information sheet (Appendix B.1), an informed consent was obtained (Appendix B.2.). Furthermore, women who gave birth to babies of over 4 kg were also not randomised. To enable this, the baby was weighed immediately after delivery. So, when the birth weight was 4 kg or less, the randomisation envelope was then opened and the study drug administered. In all cases the drug was administered within a minute of birth.

The delivery of the baby was left entirely to the midwives. Routine practice is to give the uterotonic at the delivery of the anterior shoulder and deliver the placenta using controlled cord traction. Once the baby is delivered, the cord is cut and the baby weighed immediately. All babies are then transferred to the neonatal unit for 2 hours of observation, even if the birth and immediate neonatal period were completely normal. Once the woman is transferred to the postnatal ward (after 2 hours of observation on labour ward), the baby rejoins her and she is then able to start breastfeeding. This routine was not changed for women in the study except that the study drug was given by the researcher immediately after the baby was weighed (which occurred within seconds of delivery) rather than at delivery. For women receiving misoprostol, the oxytocin was omitted.

Once the woman was determined to be eligible, the next successive treatment envelope was opened and the designated treatment given. We randomly allocated the eligible women to 200 mcg, 400 mcg or 600 mcg sublingual misoprostol (Cytotec; Pfizer, Italy). The tablets were moistened with tap water prior to being placed under

the tongue. At the same time, an 'under-buttocks drape with fluid collecting pouch' (Kimberly-Clark, Kent, UK) was placed under the woman's buttocks for collection of any blood over the next 120 minutes. Immediately after placental delivery, an intrauterine catheter (Koala External Balloon Catheter IPC-5000E, Clinical Innovations, Utah, Figure 6) was inserted manually through the cervix into the uterine cavity until the tip of the catheter could be felt to touch the fundus. The catheter was secured in place with tape to the mother's thigh and connected to a Corometrics 118 maternal / fetal monitor (Corometrics Medical Systems Inc, Texas, USA). The uterine activity was recorded and saved over the next 120 minutes. A researcher was with the women throughout the 2 hours of observation to record maternal temperature, pulse, and blood pressure before labour, immediately after the delivery and then at 30, 60, 90 and 120 minutes .The women were closely observed for any side effects experienced (Appendix B.3. Case Report Form).

Figure 6, Koala External Balloon Catheter IPC-5000E, Clinical Innovations, Utah

3.2.3. The study outcomes

a) Primary

The primary outcome was the intrauterine pressure over the first 10 minutes after the delivery of the baby. The slight differences in time during which the pressure was measured in the first 10 postnatal minutes (the catheter was only inserted after placental expulsion) meant that the Montevideo units (MVU) measurement had to be adjusted for the first reading. This was done by adding together the uterine pressures collected in the first 10 minutes from delivery of the baby, dividing it by the length of minutes during which the measurements were taken (to get MVU per minute) and then multiplying by 10.

b) Secondary

The secondary outcomes include:

- Change in the intrauterine pressure from 20 to 120 minutes using MVU.
- Side effects of treatment (Puse, blood pressure and temperature at 30. 60. 90 and 120 minutes)
- Measured blood loss in the 120 minutes after delivery
- Additional use of other uterotonic drugs to treat patients who might develop PPH.

3.2.4. Ethical consideration and consent

The study was approved by University of Liverpool Ethics Committee (RETH000237) and accepted by the local hospital committees in Zliten and Misurata Teaching Hospitals.

All subjects were informed of the nature and purpose of the study, its requirements and possible hazards, and their right to withdraw at any time from the study without prejudice and without jeopardy to any future medical care at the study site. Participants had the purpose of the study explained and received appropriate written information. All subjects were offered adequate opportunity to ask the investigator or nominated designee about any aspect of the study. The consent sought on admission

to the labour word. Each participant agreed to co-operate in all aspects of the study and gave informed written consent to the investigator for participation. They asked to sign three informed consent forms, one for the investigator and one for the hospital and one to themselves.

3.2.5. Safety Assessment

The safety of the women in this study is of high importance. Participants recruited had no invasive procedures unless the responsible clinician felt it will not affect woman's health.

Where any woman diagnosed with PPH during her participation in the study, treatment of PPH was started immediately as follows,

- Uterine massage ('rubbing up the fundus) to stimulate contractions
- Ensure bladder is empty (Foley catheter, leave in-situ)
- Syntocinon 10 units by slow IV injection
- Ergometrine 0.5 mg by slow IV injection
- Syntocinon infusion (30 units in 500 mls Hartmann's at 125 mls / hr)
- Misoprostol 600 mcg in case of continued bleeding

Any side effects or complications that developed during the study were treated and reported and recorded in the serious adverse events form.

3.2.6. Direct Access to Source Data and Documents

All information obtained as a result of the study were kept confidential and there were no disclosure to any third party other than the Ethics Committee. Records identifying the participant will be kept confidential and, to the extent permitted by the applicable laws and/ or regulations, will not be made publicly available.

Documents relating to the study that contained personal data that may disclose the identity will remain with the investigator and will not provide any personal data that may identify the participant to any third party at any time during the study.

4. Statistical analysis

a. The validity study

To test the validity of the Koala External IPC 5000E and Intran pluse IUP-500 intrauterine pressure catheters, the pressure recorded by each catheter was compared with the true pressure exerted by the sphygmomanometer or the Latitude calibrator using limits of agreement (LOA) (Bland & Altman 1986). The narrower the LOA of a catheter, the better the measurement of the intrauterine pressure.

b. RCT

The difference in means of the primary outcome was calculated using one way ANOVA. The intrauterine pressures from 20 to 120 minutes postpartum were secondary outcomes. Repeated measures of longitudinal data analysis (repeated measures ANOVA) were used to compare the effect of the study treatments over 120 minutes of observation. Other mean comparisons were done using the one way ANOVA with multiple comparisons Bonferroni test. The incidence of shivering and fever and other side effects among the three misoprostol group were compared using Chi square test with Yates' correction and Fisher exact test as appropriate. Statistical analysis was performed using Excel and PASW Statistics 17. The difference between the 4 groups' parameters was taken as statistically significant when P values were less than 0.05. Based on the primary outcomes (mean intrauterine pressure over the first 10 minutes) and to detect a significant difference (P=0.05, two-sided) at 0.8 power with 50% effect size, we needed a sample size of 12 in each group. The calculation was done by G*Power 3.0.10 software.

5 . Results

5.1. Results of the validity study

1. a. Limits of agreement of Intran catheter versus the calibrator

The mean difference in pressure values recorded by the Intran catheter and the pressure exerted by the calibrator was 0.75 mmHg (95% CI 1.1-0.42). The limits of agreement were -1.4 to 2.9.

The plot in Figure 7 shows graphically that the difference in individual pressure values obtained by the Intran catheter and that produced by the calibrator. The range of pressure values was only 4.3 mmHg.

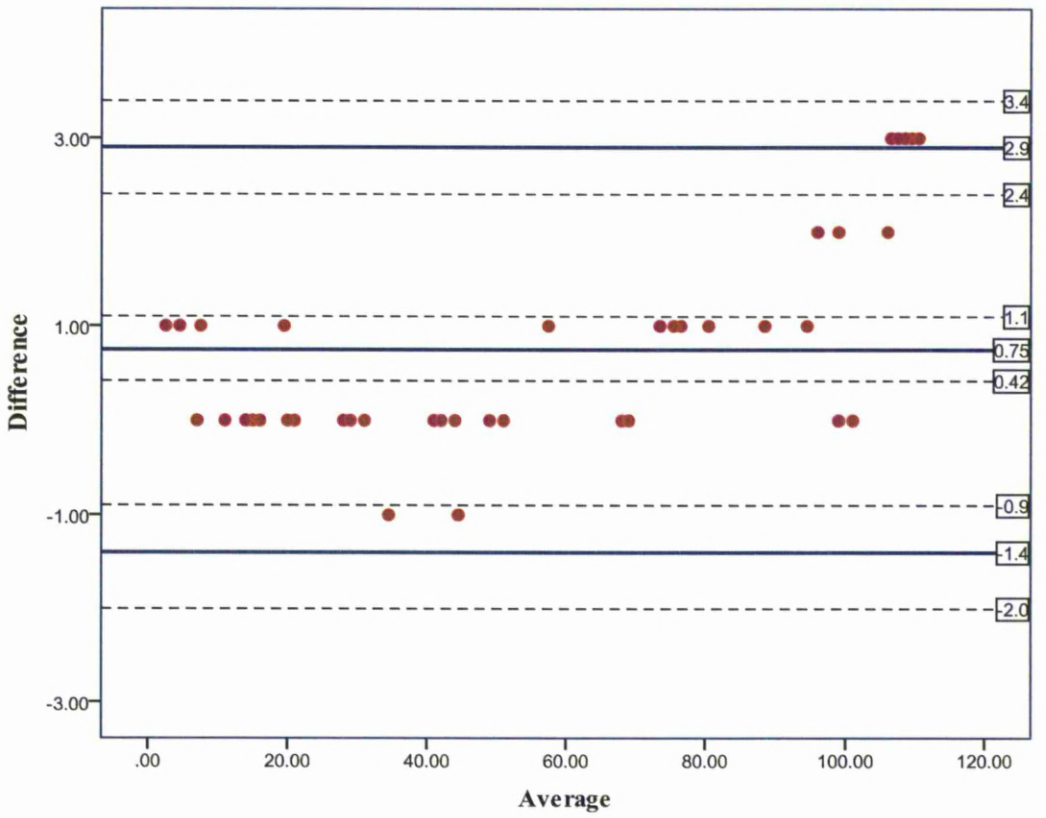

Figure 7. Difference against mean of pressure data of Intran pluse IUP-500 catheter and the Latitude calibrator

1.b. Limits of agreement of Koala catheter versus the calibrator

The mean difference in pressure values recorded by Koala and the pressure exerted by the calibrator was 2.1 mmHg (95% CI 1.26–2.94). The limits of agreement were -3.2 and 7.4 mmHg.

Figure 8 shows graphically the difference in the pressure values obtained by Koala and the actual pressure values produced by the calibrator. The range of the difference was 10.6 mmHg. The scatter plot of the raw data showed that the pressure measured by the Intran catheter is more correlated with the true pressure exerted by the calibrator than the Koala catheter (Figure 9).

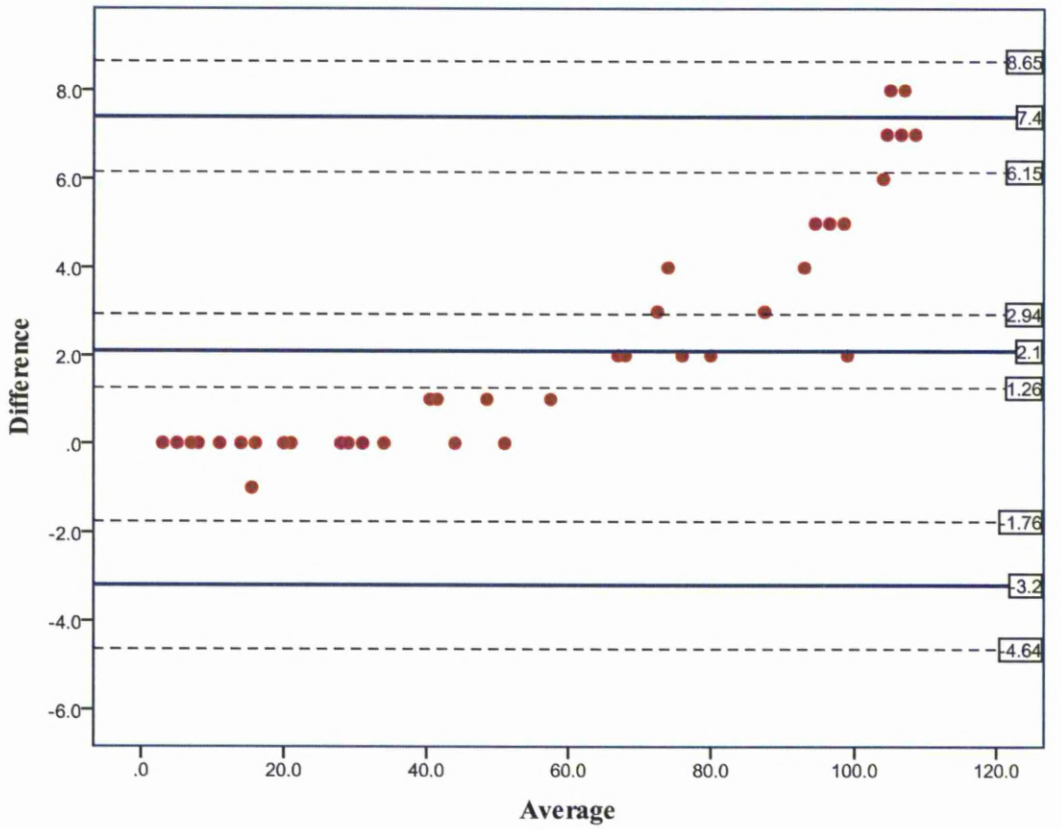

Figure 8. Difference against mean of pressure data of Koala External IPC 5000E catheter and the Latitude calibrator

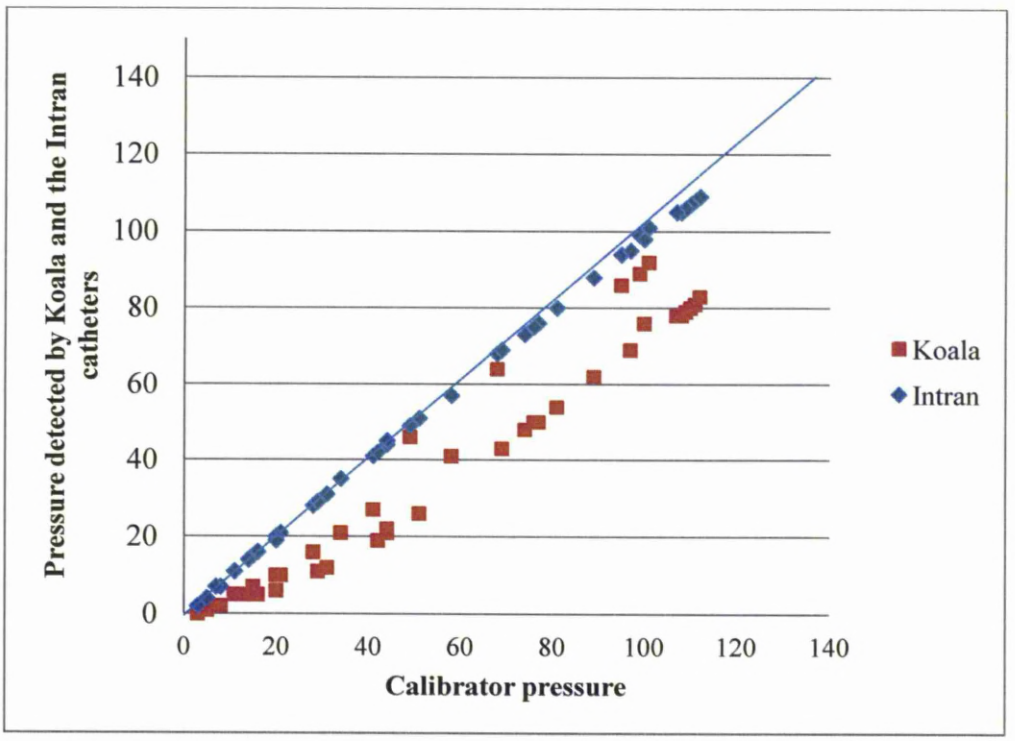

Figure 9. Scatter plot for the raw data for the pressures measured by the Intran and Koala catheters and the Calibrator

2. a. Limits of agreements for Intran (within balloon filled with water) versus sphygmomanometer

The mean difference in pressure values recorded by the Intran catheter within a balloon filled with water and the pressure exerted by the sphygmomanometer was 84.5 mmHg (95% CI 74-95). The upper and lower limits of agreements were 178.5 and -9.5 respectively.

Figure 10 shows that the range of the limits of agreement was 188 mm Hg.

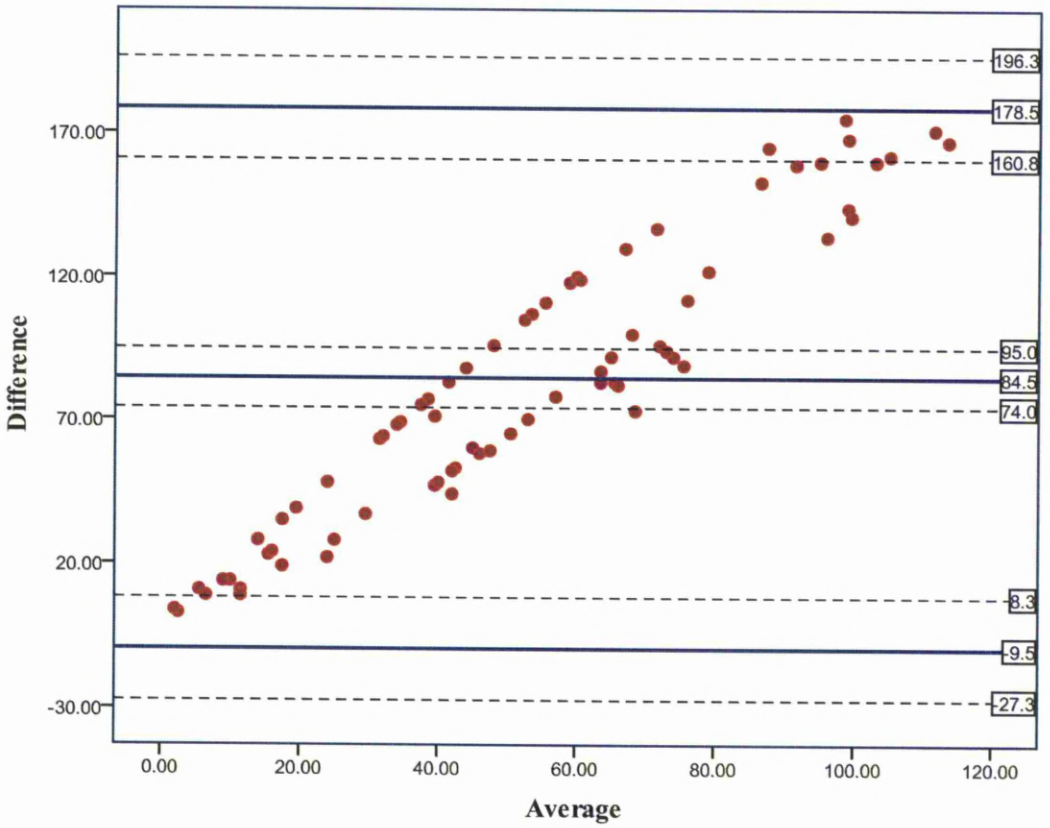

Figure 10 . Difference against mean of pressure data of Intran pluse IUP-500 catheter (within balloon filled with water) versus sphygmomanometer

2. b. Limits of agreements for Koala (within balloon filled with water) versus sphygmomanometer

Figure 10 illustrates the mean pressure difference between the Koala catheter within a balloon filled of water and the sphygmomanometer. The mean difference was 62 mmHg (95% CI 55.1–68.9). The upper and lower limits of agreement were 123.9 and 0.1 mmHg respectively.

Although the range of the difference is wide, at 123.8 mmHg, this difference was less than in the Intran catheter (188 mmHg). Neither catheters pressure showed a good correlation with the pressure exerted by the sphygmomanometer. However, the Koala catheter was slightly better than the Intran catheter (Figure 11). Furthermore, there is a clear and direct correlation between pressure and error as can be seen on the correlation graph (Figure 12) and the limits of agreement graphs (Figure 10 & 11).

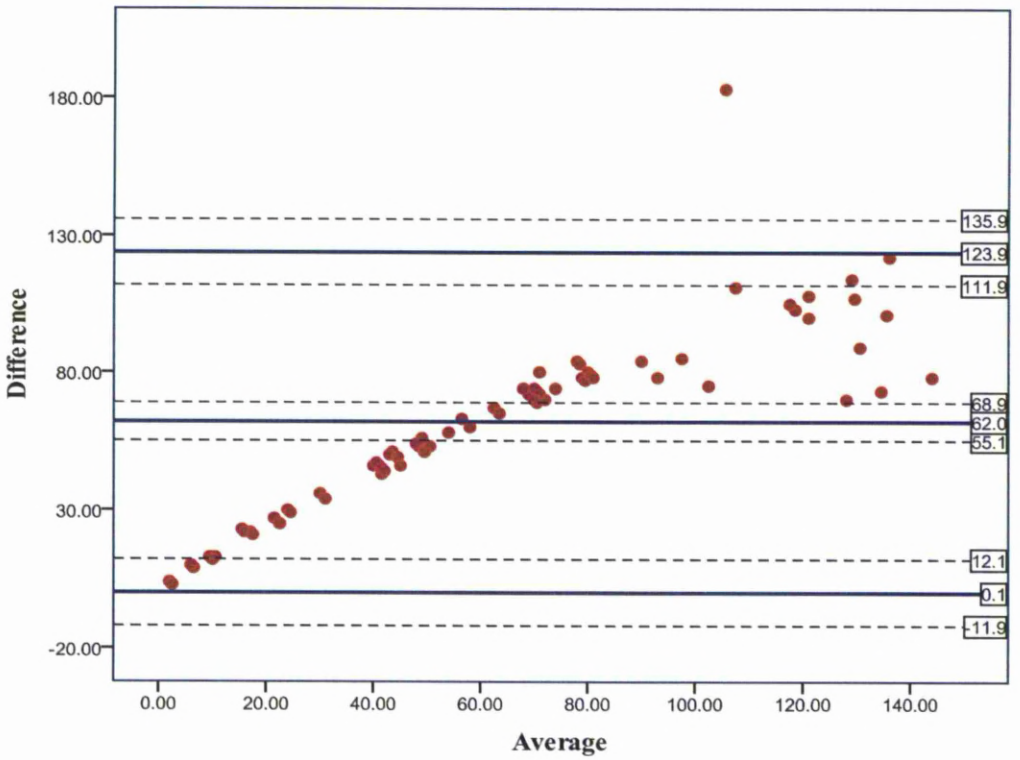

Figure 11. Difference against mean of pressure data of Koala External IPC 5000E catheter (within balloon filled with water) versus sphygmomanometer

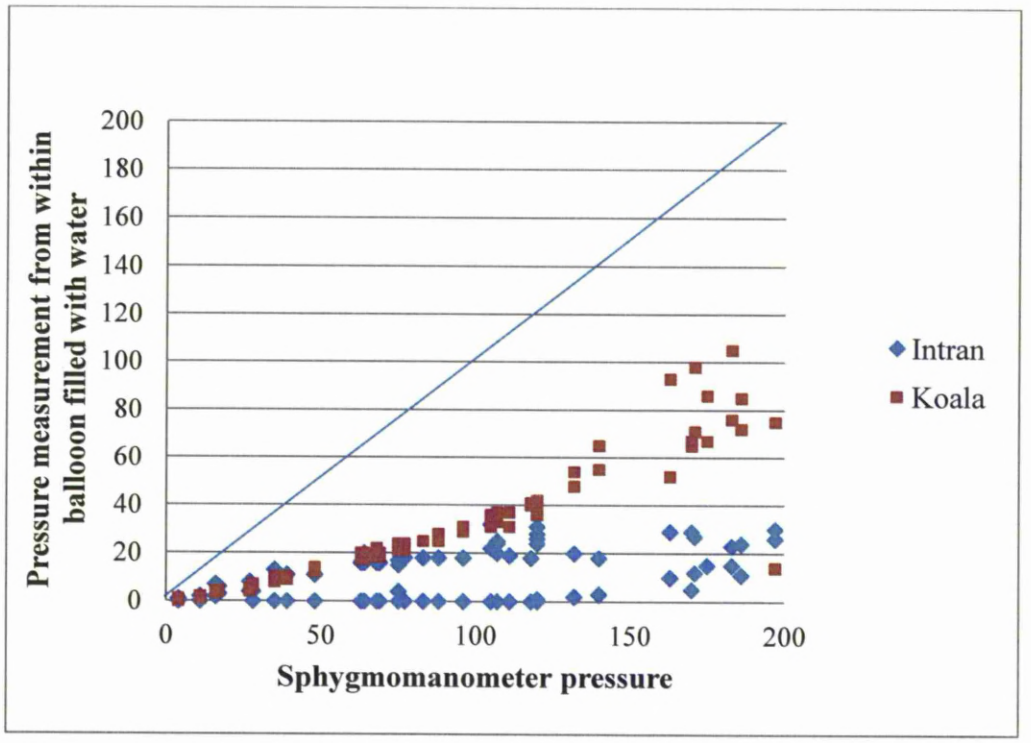

Figure 12. Scatter plot for the raw data for the pressure measurement of Intan and Koala catheters within balloon filled with water

3. a. Limits of agreements for Intran within wet tissue versus sphygmomanometer

As shown in Figure 13, the mean difference in pressure recorded by Intran catheter within wet tissue and the actual pressure exerted by the sphygmomanometer was 80.2 (95% CI 65.1–95.3). The Limits of agreements were -15.4 and 175.9 mmHg giving a range of 191.3.

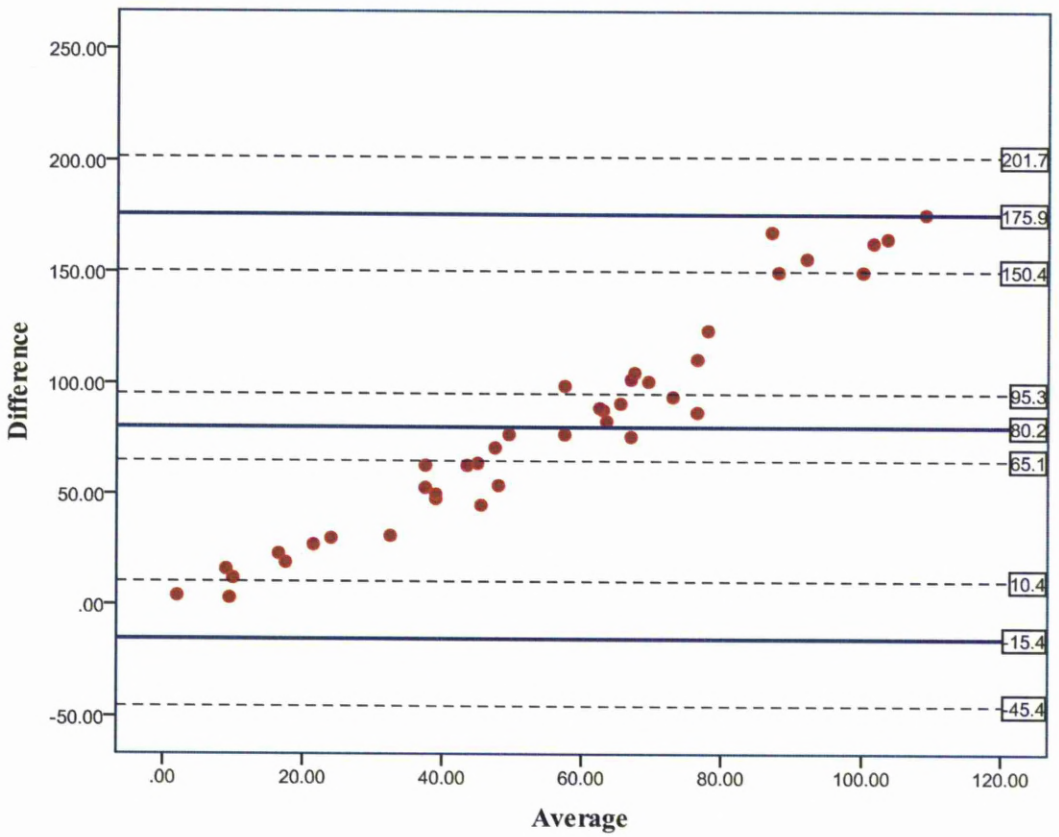

Figure 13. Difference against mean of pressure data of Intran pluse IUP-500 catheter (within wet tissue) versus sphygmomanometer

3. b. Limits of agreements for Koala within wet tissue versus sphygmomanometer

The mean difference in pressure values recorded by Koala catheter and the sphygmomanometer was 41.0 mmHg (95% CI 50.8–31.2). The limits of agreement were 19.8 and 101.8 mmHg.

As can be seen in Figure 14, the range of the limits of agreement is 121.6 mmHg. However, it is less than the range of difference recorded by the Intran catheter (191.3).

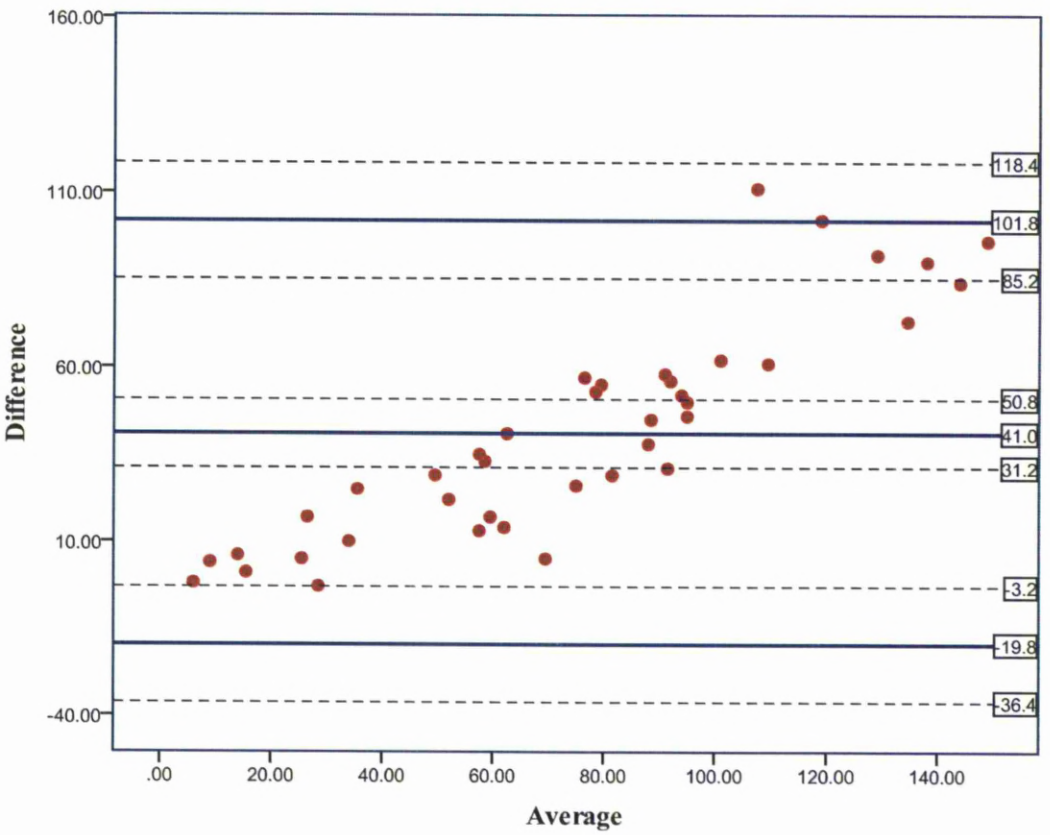

Figure 14. Difference against mean of pressure data of Koala External IPC 5000E catheter (within wet tissue) versus sphygmomanometer

Niether catheters' pressure measurements within the wet tissue showed a good correlation with the pressure exerted by the sphygmomanometer. However, the Koala catheter was better than the Intran catheter (Figure 15).

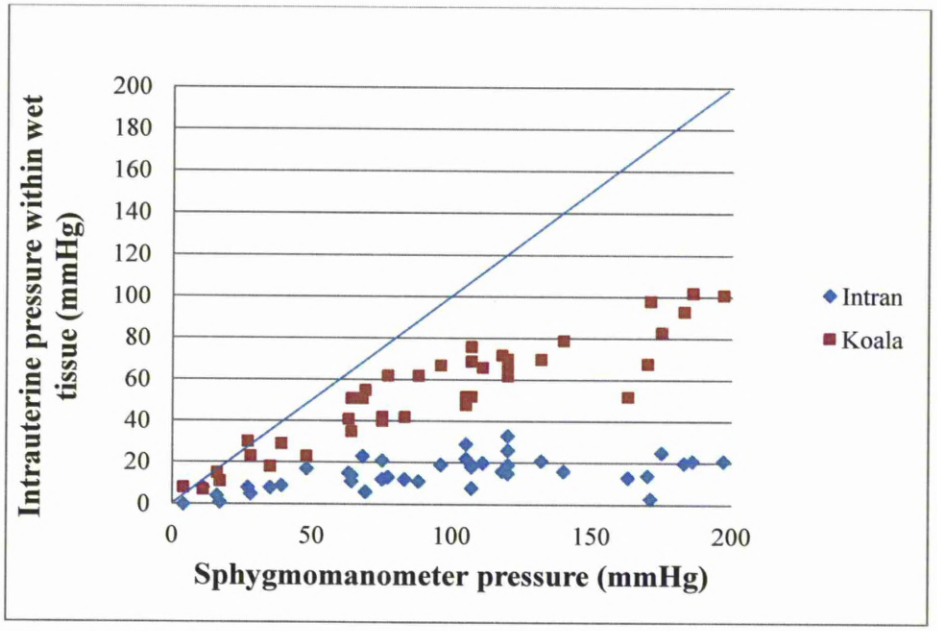

Figure 15. Scatter plot for the raw data for the pressure measurement of Intan and Koala catheters within wet tissue

5.2. Results of the randomised trials

227 were eligible to participate in the study after applying the primary selection criteria and informed about the study. 178 women were excluded because they had induced or augmented labour, caesarean section and instrumental delivery. 49 women were eligible and participated in the study (Figure 16).

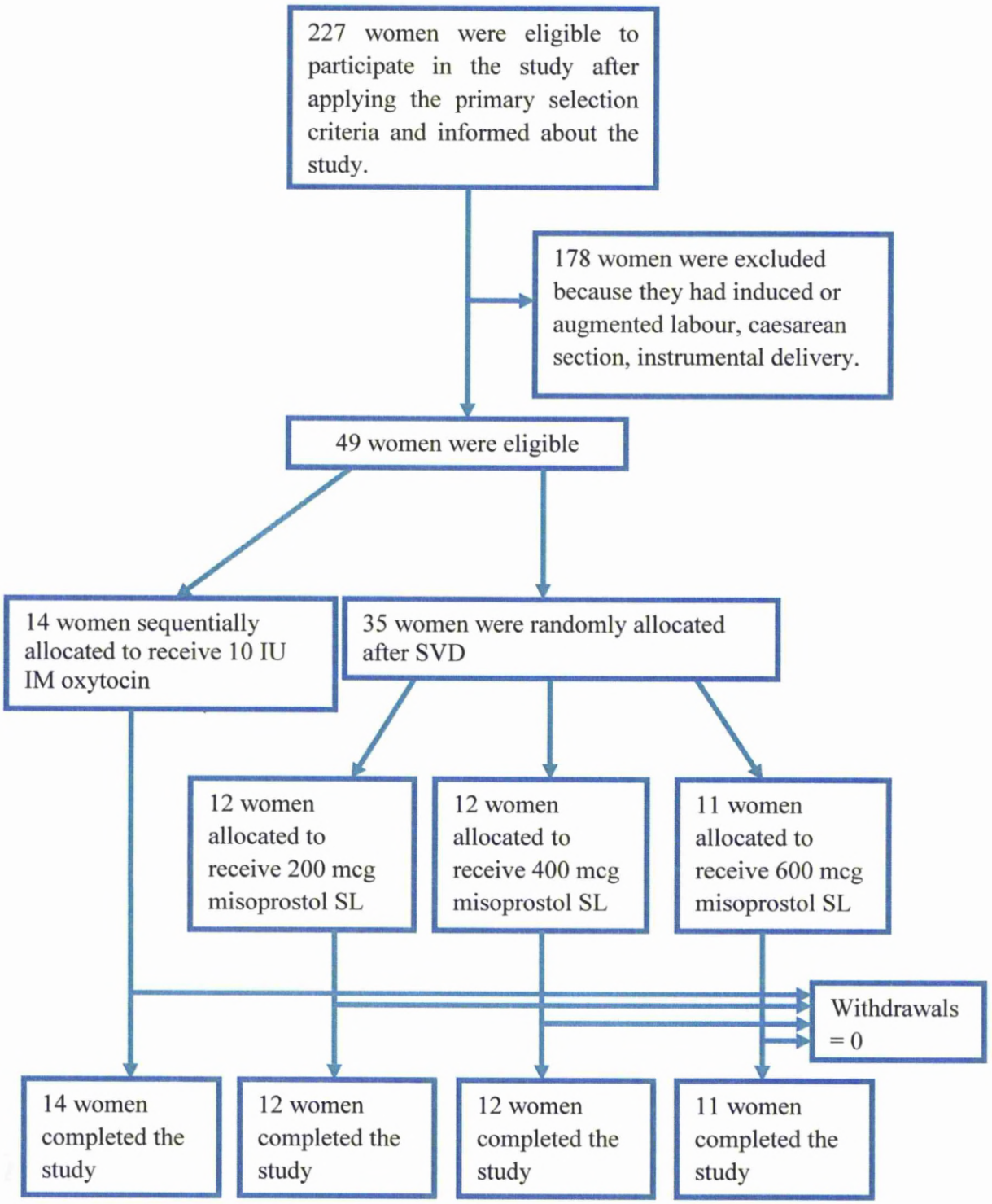

Figure 16. Flow diagram of the progress of the study

5.2.1. Basic Characteristics of the study population

A total of 35 women were randomised out of a planned sample of 36. The study was curtailed before the planned finish when I had to return to the UK. The randomised women were compared to a cohort of 14 women treated with oxytocin prior to the start of the randomised study. The age, parity, gestational age and baby's birth weight was similar in the four groups (Table 1).

Table 1. Basic characteristics of four treatment groups presented as mean ± SD

Medication Given	10 IU Oxytocin	200 mcg SL misoprotol	400 mcg SL misoprostol	600 mcg SL misoprotol
No of women recruited	14	12	12	11
Parity (n/%)				
0	2 (14%)	0	1 (8.3%)	1 (9%)
1	4 (28.5%)	3 (25%)	3 (25%)	2 (18%)
2	4 (28.5%)	5 (41.5%)	4 (33.3%)	5 (45%)
>2	4 (28.5%)	4 (28.5%)	4 (28.5%)	3 (27.3%)
Age (Years)	28.5 ± 6.2	28 .1 ± 5.4	27 ± 3.3	26.3 ± 3.7
Gestational age (weeks)	40.2 ± 1.4	40.1 ± 1.1	40.3 ± 0.8	41 ± 1.1
Birth weight (gm)	3350 ± 0 364	3458 ± 0 262	3595 ± 0 397	3522 ± 0 337

5.2.2. Effect of oxytocin and three sublingual doses of misoprostol on the intrauterine pressure

All three doses of sublingual misoprostol showed a rapid increase in uterine activity with a peak at 40 minutes followed by a gradual decrease over the following 80 minutes (Figure 17). Throughout the time of observation, there was no significant difference between the intrauterine pressure in the three misoprostol dosage groups (P=0.8).

The uterine pressure in those receiving oxytocin was highest in the immediate postpartum period and declined gradually after 40 minutes of administration. In the first ten minutes, intra uterine pressure of the three misoprostol groups was significantly lower than that of the oxytocin group (P=0.008). Conversely, the uterine pressure over the period from 50 to 120 minutes was significantly higher in the three misoprostol groups than in the oxytocin group (P=0.008).

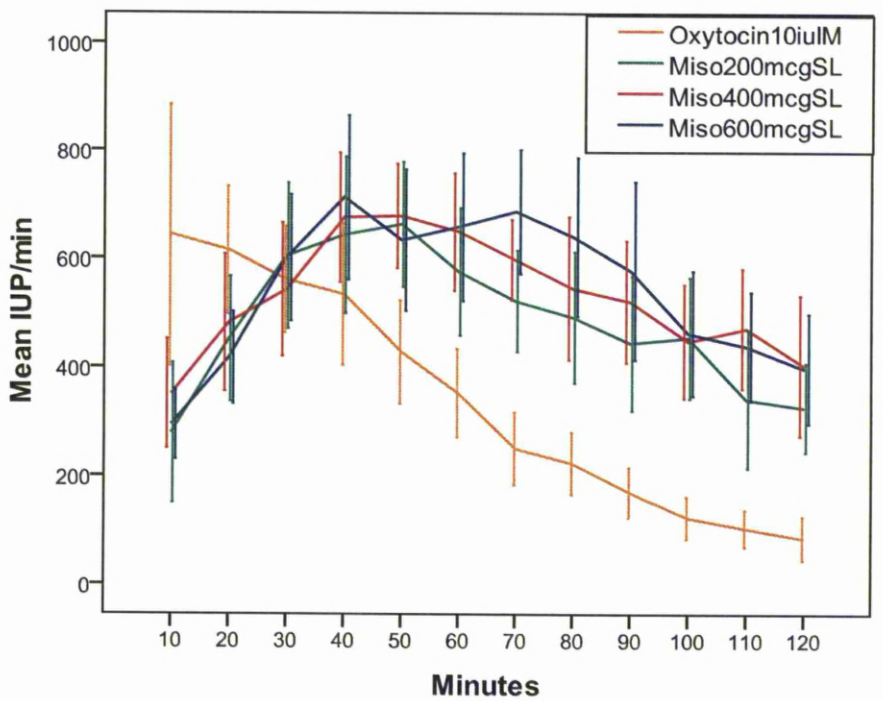

Figure 17. Mean postpartum uterine pressure in the different treatment groups. Data is presented as Montevideo units (±SEM). The data in the first 10 minutes is adjusted to correct for the timing of placental delivery (MVU per minute).

5.2.3. Side effects of oxytocin and misoprostol

All the women were closely observed for side effects. As shown in Table 2, women who received intramuscular oxytocin reported no side effects. In the misoprostol groups, the two most commonly reported side effects were shivering and hyperpyrexia, and a dose-related rise in the body temperature was observed. The incidence of severe hyperpyrexia (>39°C) was higher in the 600 mcg misoprostol group than the 200 mcg and 400 mcg misoprostol groups, but this was not statistically significant (P=0.1; Figure 4). Most of the women were not aware of the fever, but complained of coldness and marked shivering. All women in the 400 mcg and 600 mcg groups had shivering compared to 75% of the women in the 200 mcg misoprostol. The differences were statistically significant (P=0.04).

Table 2. Incidence of side effects in the different treatment groups. Number (%)

Medication Given	10 IU Oxytocin	200 mcg SL misoprotol	400 mcg SL misoprostol	600 mcg SL misoprotol
No of women recruited	14	12	12	11
Temperature >39°C	0	1 (8.3%)	1 (8.3%)	5 (45%)
Chills / shivering	0	9 (75%)	12 (100%)	11 (100%)
Coldness	0	9 (75%)	12 (100%)	11 (100%)
Abdominal pain	0	0	2 (16.6%)	0
Nausea & vomiting	0	0	2 (16.6%)	1 (9%)
Skin rash	0	0	0	1 (9%)

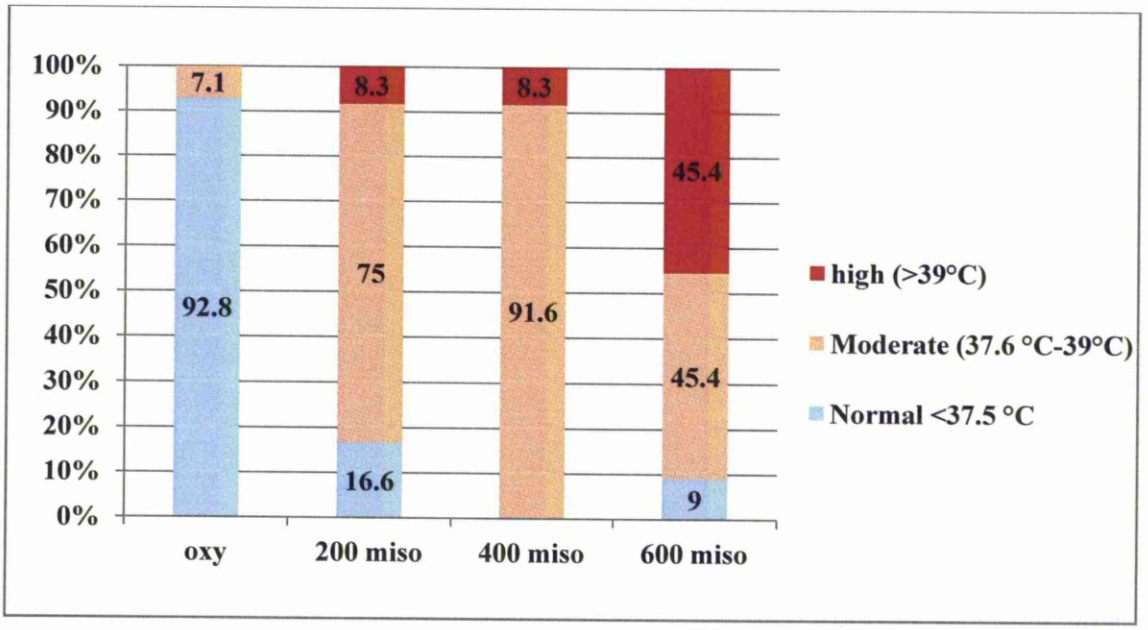

Figure 18. The incidence of fever in 10 IU oxytocin IM, 200 mcg, 400 mcg, 600 mcg sublingual misoprostol

5.2.4. Blood loss

The blood loss was under 500 ml in all women and was normally distributed. The lowest mean blood loss (±SD) was observed in women who received 10 IU oxytocin (193±35 ml), followed by those who received 200 mcg SL misoprostol (239 ± 46 ml), 600 mcg SL misoprostol (275 ± 62 ml) and 400 mcg SL misoprostol (299 ± 60 ml). The difference was statistically significant between oxytocin and 400 mcg misoprostol (P<0.001), oxytocin and 600 mcg misoprostol (P<0.001) and also between the three misoprostol groups (P=0.04). Howevere, the study was not powered to detect difference in blood loss. None of the women received additional uterotonics or blood transfusion.

5.2.5. Additional analysis of the study data (post-hoc)

This additional analysis was carried out to analyse in depth and separately the role of uterine strength and uterine frequency in the mechanism of cease postpartum bleeding.

5.2.5.1. Effect of different treatments on uterine contraction strength

The intrauterine pressure was considered here to measure the strength of the uterine contractions. The graph shows the mean intrauterine pressure for the 4 treatment groups (Figure 19). Over the first 20 minutes post partum, all the investigated treatments showed non-significant difference of the intrauterine pressure ($P>0.05$). Thereafter, the mean intrauterine pressure of the three different sublingual misoprostol doses (200 mcg, 400 mcg and 600 mcg) was higher than the IUP of the 10 IU oxytocin ($P<0.05$).

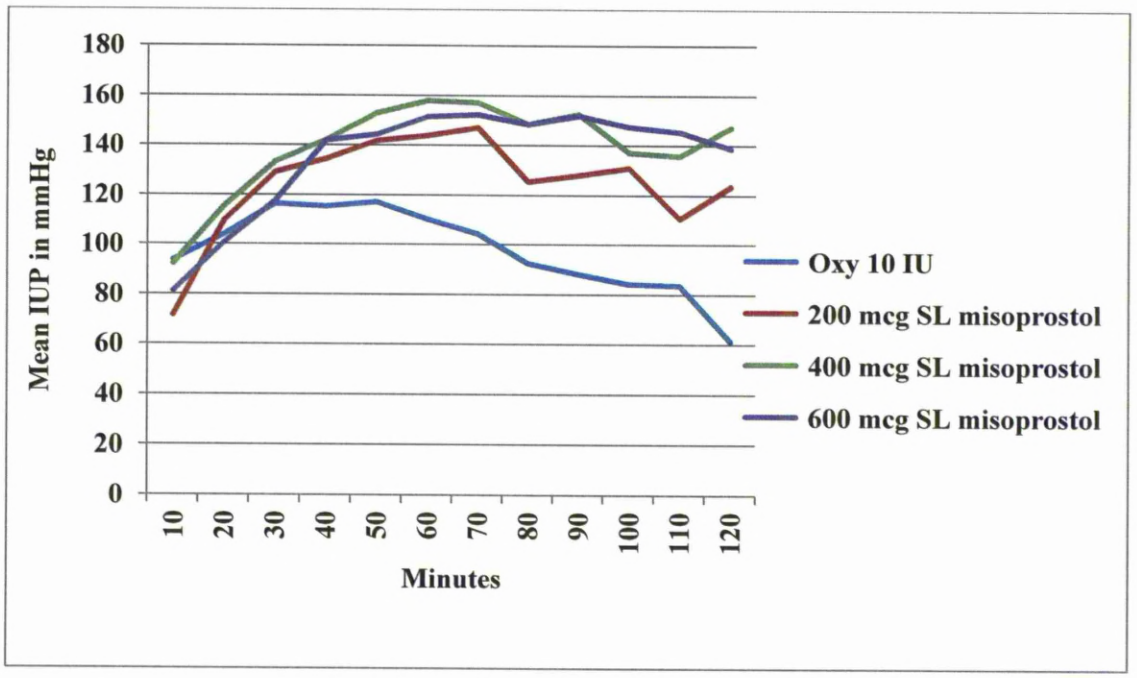

Figure 19. Mean intrauterine pressure in different treatment groups (10 IU oxytocin, 200 mcg, 400 mcg, 600 mcg sublingual misoprostol)

5.2.5.2. Effect of different treatments on uterine contraction frequency

Figure 20 shows the mean frequency of uterine contractions in the different treatment groups over 120 minutes. Oxytocin had a significant effect on the uterine contraction frequency compared to the three sublingual doses of misoprostol over the first 10 minutes postpartum ($P<0.05$). Over the next 30 minutes, there was non-significant difference in the uterine contraction frequencies between the treatments ($P>0.05$). Thereafter, misoprostol had a higher frequency of uterine contractions compared to oxytocin ($P< 0.05$).

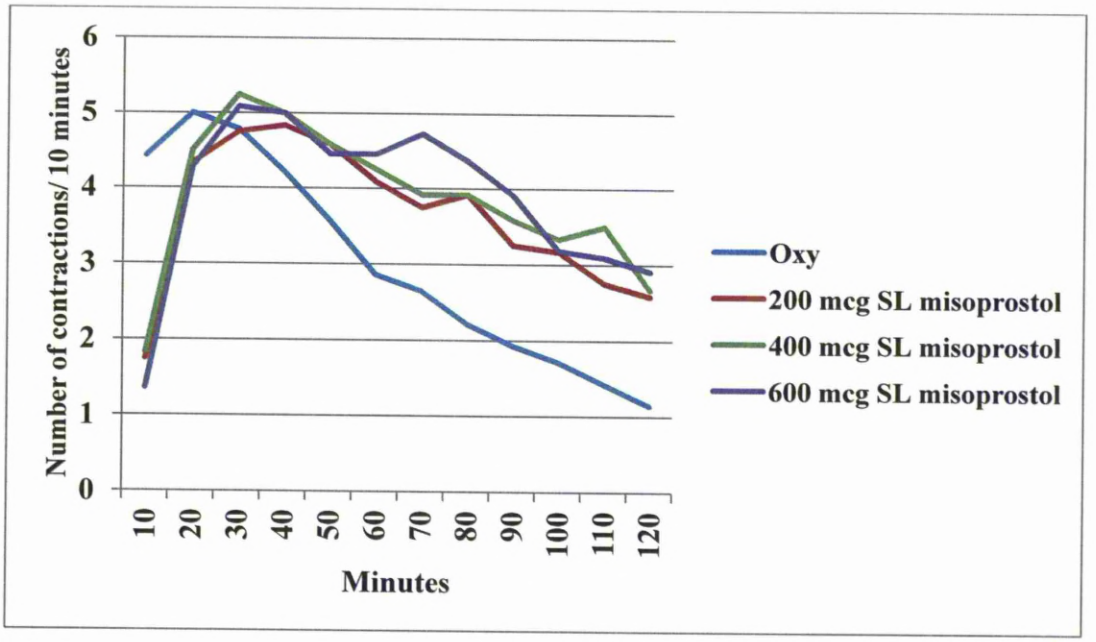

Figure 20. Mean uterine contraction frequency in different treatment groups (10 IU oxytocin, 200 mcg, 400 mcg, 600 mcg sublingual misoprostol)

6. Discussion

This study was conducted to find out whether high dose misoprostol offered any significant improvements in postpartum uterine pressure over low dose misoprostol, and to compare the adverse drug reactions associated with each treatment. We also had concurrently collected data on intrauterine pressures following oxytocin, and this allowed us to compare the IUP produced by these two types of uterotonics. For the measurements of IUP postpartum, we examined the validity of two types of IUP catheters to choose the best of them for the trial.

The validity experiment has shown that both the tip transducer (Intran) and external transducer (Koala) catheters are valid and sensitive to changes in the surrounding pressure when they are tested inside the calibrator. The limits of agreement were very close and the differences were not clinically significant for either type of catheter. This clearly demonstrates that both catheters are working effectively when they are tested in an optimum situation. The closed system in which they are surrounded by air transmits the pressure equally and consistently to the membrane in the tip transducer of the Intran catheter and to the balloon sensors on the tip of the Koala catheter.

Neither catheter is designed to measure the pressure exerted by a flat surface as occurs for intrauterine pressure postpartum – they are mainly produced to measure the intra-partum intrauterine pressure in the amniotic cavity. Therefore, some inconsistency and inaccuracy in the pressure values was expected when using them within the wet tissue. But it should be accurate in a fluid filled cavity when using the balloon filled with water. However, that was also similarly inconsistent and inaccurate. This may be due to lack of sensitivity in the balloon wall as it is flexible and so the pressure was not transmitted correctly from the sphygmomanometer cuff to the water to the sensors. The limits of agreement of both types of catheters were very wide and the difference was significant. This may show that using these catheters in a suboptimum situation will carry a high risk of inaccuracy. However, there are no catheters specifically designed for the measurement of intrauterine pressure postpartum and the methods we used in trying to mimic the third stage of labour may not be the optimum. On the other hand, the wide variability in the results of both catheters may be related

to the fact that they are not designed to work outside a fluid cavity. Conducting this experiment in vivo would be the best method. However, this was not possible as there is no way of knowing the 'true' postpartum pressure.

There was an in vivo study conducted to test the reliability of the Gaeltec catheter-tip pressure transducer for the measurement of intrauterine pressure in the third stage of labour (Chua et al., 1998). This study compared the reliability when two catheters were connected together and when they were inserted independently into the uterus. Comparison of pressure reading from both catheters revealed good agreement whether they were tied together or were separate. This means that the Gaeltec catheter-tip pressure transducer is reliable in detecting the uterine pressure at any site in the uterus. But their accuracy was not demonstrated and given that the sensor membrane was at the end of a narrow channel, it was likely to be very low. Therefore, in our experiment we tried to find a different type of catheter to use for the measurement of intrauterine pressure postpartum. Koala catheter was a good candidate as it has two inches balloon of sensing membrane surrounding its tip, which provides a wide area to be in contact with the uterine wall compared to a pin-point membrane at the tip of the Intran catheter. In our two methods to mimic the postpartum situation, the the accuracy of the Koala was better than that of the Intran, but was still poor.

The reliability of a clinical test means a consistency in the measurements produced by that test at different time points by the same observer (intra-observer reliability) and the consistency of the measurements between two observers (inter-observer reliability). However, all the measurements may be not accurate when compare with the true state which may be obtained by using a reference standard or "gold standard" (Khan & Chien 2001). When the accuracy is poor, it is not valuable to measure inter or intra observer reliability.

In the randomised trial, we utilised the Koala External Balloon senor system (Dowdle 1997; Dowdle 2003) for the measurement of the uterine activity (Figure 6). The mean intrauterine pressure over 120 minutes for the intramuscular oxytocin and the three doses of sublingual misoprostol were shown in Figure 17. The difference seen in speed of onset of action can be explained both by their differing pharmacokinetics and by their different routes of administration. The absorption of misoprostol is affected

by mouth dryness and any surrounding fluid, (Singh et al., 1999; Tang et al., 2002) but we standardised for this by moistened the tablets with tap water before placing them under the tongue. The pharmacokinetics may explain our observation of stronger and more frequent initial uterine contractions with intramuscular oxytocin than with sublingual misoprostol. The peak of uterine contraction for oxytocin was within the first 10 minutes post administration whereas for sublingual misoprostol it was only achieved after 30 to 40 minutes. This mirrors their plasma concentrations in the previous studies (Tang et al., 2002; Weeks 2006).

Most uterine bleeding occurs immediately after placental separation (Hytten 1995). At this time the mean intrauterine pressure of intramuscular oxytocin was significantly higher than with the three sublingual misoprostol doses. Even though the effective action of sublingual misoprostol started late, it caused high uterine contractions maintained over a considerable period. This may help to prevent steady moderate bleeding which may be unobserved until serious hypodynamic manifestations occur.

Very few studies have examined the effect of misoprostol on the uterine activity during the third stage of labour using measurement of intrauterine pressure as an indicator. Chong and collegaues using a Galtec tipped catheter, found no difference in postpartum uterine pressures between oral misoprostol and intramuscular syntometrine (Chong et al., 2001).

The mean intrauterine pressure of the three different doses of sublingual misoprostol was not significantly different over the two hours observation period. This is consistent with previous research findings. For example, in one previous study there was no difference seen in intrauterine pressure measurements with 5 different doses of oral misoprostol, although again this used a Galtec tipped catheter (Chong et al., 2001). Furthermore, randomised control trials found that both 600 mcg sublingual and 400 mcg oral misoprostol were more effective than placebo for prevention of PPH (Hofmeyr et al., 1998; Hoj et al., 2005) and a meta-analysis in which an indirect comparison was made between the 400 and 600 mcg oral doses concluded that the two were likely to be equally effective (Hofmeyr & Gulmezoglu 2008). As a result of side effects associated with high doses of sublingual misoprostol, some researchers have therefore argued that the dosage of misoprostol should be reduced from the 600

and 800 mcg doses in common usage today. Our research findings give further weight to that argument.

The most commonly observed side effects in this study were shivering and fever. Around half of the women who had 600 mcg had a temperature >39°C whereas the incidence for women who received 400 mcg and 200 mcg was around 8%. Most of the women were not aware of the increase in their body temperature, but complained of coldness and intolerable shivering (all women in the 400 mcg and 600 mcg groups had shivering). Although there appeared to be clinical differences in the incidence of fever between high and low doses, the difference was not statistically significant as the study was not powered enough to detect differences in incidence of side effects. Shivering and fever have been reported in most of the studies using misoprostol for different indications and with variable routes and doses. In a recent multicentre randomised control trial using 800 mcg sublingual misoprostol for treatment of PPH, the overall incidence of fever (>40°C) was 14%. However, the incidence of fever varied between different populations with the highest incidence in Ecuador (36%) and the lowest (0%) in Egypt (Winikoff et al., 2006; Winikoff et al., 2010). In our study, the incidence of side effects appeared to be dose–related. Prostaglandins are the principal mediator of fever in the brain and can pass the blood brain barrier to the thermoregulation centres in the hypothalamus, causing elevation of the thermoregulatory set-point. In order to increase the body temperature to the new set-point, there are increases in heart rate, muscle tone and shivering (Boulant 2000; Kanosue, Yanase-Fujiwara & Hosono 1994; Kanosue et al., 1994).

Clinical trials have shown that oxytocin prophylaxis is more effective than oral misoprostol for the prevention of blood loss over 1000 mls (Gulmezoglu et al., 2001) . Given that most blood is lost around the time of placental expulsion during the first 10 postpartum minutes, it is not surprising that oxytocin is the more effective prophylactic (Weeks 2006). It is only after the first 50 minutes that the uterine contraction strength was higher in the misoprostol groups. However, although the effect of misoprostol is delayed, it should be eventually as effective as oxytocin in preventing massive blood loss through atony. There is evidence for this from the Cochrane review in which there are significant differences between oxytocin and

misoprostol in blood loss over 500 mls and 1000 mls, but no difference in need for blood transfusion (Gulmezoglu et al., 2007).

We acknowledge that this study recruited only low risk women in whom the chance of developing an atonic PPH was small. However, even in a study which included high risk women, the majority of women would not develop an atonic PPH – and this is the very group for which the intervention is designed. However, the area of interest in this study is the comparison between drug dosages and, as such, it is important to have as homogeneous group of women as possible. This was best achieved through the use of a low risk group. Furthermore, it was considered by the ethical committee to be inappropriate to expose women at risk to misoprostol which has been shown to be less effective for prophylaxis than oxytocin.

7. Post –hoc discussion

During the immediate 10 minutes postpartum, oxytocin produced similar uterine pressure (uterine contraction strength) as the three different doses of misoprostol. But, it produced more frequent contractions. This may explain the superiority of oxytocin for management of PPH. The results also prove that strength of the uterine contractions and its frequency are both essential to cease bleeding postpartum particularly during the immediate postpartum period and maintain gradual uterine involution thereafter.

Measurement of the uterine pressure is not very reliable as the intrauterine pressure catheters are mainly devised to work in the amniotic cavity. The use of intrauterine pressure as a surrogate to measure the effect of uterotonics on the uterine muscle could only help to detect differences between the treatment groups providing standardising the method for each treatment arm. Uterine contractions and its frequency can be detected easily and correctly either clinically or by intrauterine catheters. In this study we found that the trend of the effect of the treatment groups' on uterine frequency (Figure 20) is similar to the trend of the effect measured using MVU which involves both uterine pressure and frequency (Figure 17). This may suggest the possibility of using the uterine frequency as a valid parameter to measure

the effect of uterotonics on the myometrium rather than the unreliable intrauterine pressure measurements.

8. Conclusion

Both Intran and Koala catheters measurements appear to be accurate in an optimum situation. However, both catheters showed wide range of limits of agreement when used in situations that mimic the intrauterine space both antenatally and postpartum. The Koala catheter was however more accurate. The Koala catheter was therefore chosen in our study for comparing the effect of different doses of sublingual misoprostol on the intrauterine pressure postpartum.

Our results showed that 200, 400 and 600 mcg doses of sublingual misoprostol produced similar levels of IUP, but that the severity of side effects was dose related. These findings suggest that lower doses of misoprostol may be as effective as high doses. Clinical applications of low doses of sublingual misoprostol for prevention of PPH should be further explored by large randomised trials comparing the effectiveness and the safety of low doses of sublingual misoprostol.

Chapter 4

Candidate gene association study of misoprostol-induced fever

1. Introduction

Although misoprostol has been demonstrated as a safe and effective alternative treatment option for PPH, concerns remain about its side effects profile. Chills and fever are considered as the commonest adverse drug reactions in some populations. In several studies, misoprostol has been associated with fever greater than 40.0°C when used for the management of PPH. One case in particular – which called the medical community's attention to alarming complication – involved a reported peak temperature of 41.9°C following administration of 800 mcg oral misoprostol prophylaxis (Chong, Chua & Arulkumaran 1997).

A PPH treatment trial in South Africa reported three women with temperature above 40.0°C following 1000 mcg misoprostol (administered 200 mcg oral, 400 mcg sublingual and 400 mcg rectal) (Hofmeyr et al., 2004). More recently, one hospital in Quito, Ecuador, documented a higher-than-expected rate of elevated body temperature ($\geq$ 40.0°C) in which 35% of all women receiving misoprostol treatment (800 mcg sublingually) experienced high fever, compared with 0–10% in the eight hospitals following the same treatment protocol (Winikoff et al., 2006). All of these cases resolved with anti-pyretic treatment and cool compresses within several hours and without complications. Nonetheless, it is unclear why some women develop high body temperature while others do not and why most high fevers occurred in Ecuador. Hence, the possibility of a genetic cause might answer this question as individuals' variations in response to medications may be caused, at least in part, by differences in their genetic profile. Pharmacogenetic is a study of the genetic basis of differences in drug response between individuals. It can involve studying DNA sequence related to drug response or by investigating the variability of the expression of individual genes relevant to disease susceptibility as well as drug response at cellular, individual or population level (EMEA 2002).

Pharmacogenomic research strategies involve identification and evaluation of genetic polymorphisms in individuals and the establishment of definitive relationships between these genetic variations and drug responses (McLeod & Evans 2001). To investigate why misoprostol-induced fever was clustered in Quito, Ecuador,

environmental factors such as Quito's high attitude, and patient characteristics such as genetic make-up of participants, have been considered. Misoprostol has been used in other high altitude settings for PPH (e.g. Pakistan) (Mobeen et al., 2011); however, similar trends in elevated body temperature have not been reported as the incidence of fever was 4%. We therefore hypothesise that some women may be more susceptible to experiencing elevated body temperature than others due to genetic predisposition, which may affect the response to misoprostol and the susceptibility of women to adverse drug reactions.

The effectiveness of misoprostol is well established in preventing PPH after vaginal delivery (Derman et al., 2006; Enakpene et al., 2007) and has been evaluated in several trials, including two large multi-centred RCTs, for its potential as an alternative therapy option for PPH . Findings from the two recently conducted RCTs have demonstrated that 800 mcg of misoprostol, when given sublingually, is similar in effectiveness to IV oxytocin in controlling excessive postpartum bleeding (Blum et al., 2010; Winikoff et al., 2010). Anecdotal evidence also suggests that misoprostol is widely used off-label by health care providers for PPH and other reproductive health indications. To date, there has been no evidence that use of misoprostol for this indication poses undue risks to women (Abdel-Aleem, El-Nashar & Abdel-Aleem 2001; Adekanmi et al., 2001; Gulmezoglu et al., 2004; Hofmeyr et al., 2001a; Kulier et al., 2004; Lokugamage et al., 2001; Ng et al., 2001; O'Brien et al., 1998; Oboro, Tabowei & Bosah 2003; Ozan et al., 2000). The research ethics committees at the University of Liverpool and in Ecuador approved the study.

The aim of this study was to identify the genetic factors involved in the development of misoprostol-induced fever in women using misoprostol in different populations. We studied misoprostol-induced fever in two populations: one in Ecuador, where misoprostol was given for treatment of PPH, and in Liverpool, United Kingdom, where women were administered misoprostol for termination of pregnancy (TOP).

2. Study design and participants

2.1. Ecuador population

The Ecuadorian population was part of a clinical trial which was designed for the assessment of fever after misoprostol administration for the treatment of primary PPH.

The clinical trial was designed to investigate whether a lower, 600 mcg dose of sublingual misoprostol, compared to the previously used 800 mcg, will reduce the incidence of elevated body temperature (≥ 40°C) associated with misoprostol. The study design also sought to document the acceptability of 600 mcg sublingual misoprostol for PPH treatment. In addition, blood samples were collected to investigate whether some women are more susceptible to experiencing high fever following misoprostol administration, and whether genetic factors are responsible for misoprostol-induced fever.

2.1.1. Description of research and samples collection

The trial has evaluated side effects after misoprostol as a first-line treatment for PPH due to suspected uterine atony. Women who were given oxytocin prophylactically during the third stage of labour were consented and had their postpartum blood loss measured following vaginal delivery (Appendix C. 1. Patient information sheet and consent form). Women diagnosed with PPH that the clinical care team deemed would benefit from uterotonic therapy to control their excessive bleeding were enrolled in the study and were given the study treatment. The treatment regimen consisted of three 200 mcg (600 mcg) tablets of misoprostol given sublingually. Delivery attendants documented the occurrence of any side effects experienced following PPH treatment. Body temperature was measured systematically using an oral mercury thermometer at 60 minutes and 90 minutes for all women given 600 mcg of sublingual misoprostol (Appendix C. 2. Blood sample/case review form). Women who had body temperature measuring ≥ 40.0°C were closely observed and had their body temperature measured and documented every half-hour until fever subsided (measuring below 38.0°C). All women had a blood sample collected (one sample of 9

ml) by venepuncture conducted under aseptic technique. The blood samples were stored at the hospital in Ecuador at -20°C and sent to the University of Liverpool in the UK for genotypic analysis to determine whether genetic factors are responsible for misoprostol-induced elevated body temperature. All samples were identified only by a code number, and could not be traceable to any individual participant.

2.1.2. Population and research location

Ecuador (Isidro Ayora Maternity Hospital)

Isidro Ayora is located in Ecuador's capital city, Quito, and attends roughly 11,000 births per year. As a large, public hospital, they often receive referrals; however, they are also linked with two local hospitals (Eugenio Espejo and Red Cross Hospital) to which they refer highly complicated obstetric cases for intensive care. Misoprostol is used regularly in Isidro Ayora for a variety of indications, including intrauterine fetal death (IUFD), treatment of incomplete abortion, treatment of PPH (when standard oxytocics fail), and cervical ripening.

Sample size calculation

Systematic measurement of elevated body temperature following misoprostol treatment served as the primary outcome measure. Any elevated body temperature measuring greater than or equal to 40.0 °C was categorized as a 'high fever.' We hypothesize that significantly fewer women will experience high fever following a 600 mcg regimen of sublingual misoprostol, compared to a previously documented rate of 36% following 800 mcg in Quito, Ecuador. It is a hypothesized that a lower dose of misoprostol will reduce the rate of high fever by 50% in this setting. To detect a one-sided difference of 50% or less, with 80% power at the alpha=0.05 level, a maximum of 75 cases of PPH was required.

A PPH rate of 30% was previously documented in this setting via objective assessment of postpartum blood loss among patients not given oxytocin prophylactically during the third stage of labour. The study hospital now routinely practices the active management of third stage of labour, which has been shown to reduce the rate of PPH by 60-75%. We therefore expect to document a rate of PPH between 8% and 12%. Assuming a 10% incidence of PPH in this setting, the samples

that were needed to be enrol approximately 750 delivering women. From the 750 women, 50 women had PPH and treated with 600 mcg sublingual misoprostol.

2.1.3. Eligibility criteria

All women presenting for vaginal delivery and who receive oxytocin prophylactically during the third stage of labour were eligible for participation. Informed consent was obtained upon presentation at the hospital. However, only women experiencing PPH and for whom uterine atony is a suspected cause were enrolled in the trial. If the provider felt that the woman, at presentation to the delivery ward, was too far advanced in her labour to give appropriate informed consent, or if she was unable to give informed consent for another reason such as mental impairment, she was not eligible for enrolment. Additional inclusion and exclusion criteria are described below.

Women's willingness to participate in the trial, to have their blood drawn, to respond to a short questionnaire to document their background characteristics and to assess acceptability of the drug regimen were also criteria for eligibility. If, at any point, the woman indicated that she no longer wished to participate in the study, she was no longer considered a participant.

2.1.4. Inclusion criteria

Women delivering vaginally and experiencing primary PPH due to suspected uterine atony were enrolled in the trial. Primary PPH was defined either through visual estimation by the provider, or as blood loss $\geq$ 700 ml using a calibrated receptacle (whichever occurred first).

2.1.5. Exclusion criteria

Women were excluded from participation if they had a known allergy to misoprostol or other prostaglandins, were not given oxytocin during the third stage of labour and/or underwent a caesarean section during the current delivery.

2.1.6. Ethical considerations

Ethical approval was sought from the Research and Ethics Committee in Hospital Gineco-Obstétrico Isidro Ayora, Quito, Ecuador. In addition, approval for the study was sought from the Liverpool University Ethics Committee where the blood samples were to be analysed. The potential risks for women who participated in the trial were the possible side effects of misoprostol, including chills, fever, shivering, painful uterine contractions and gastrointestinal upset. These side effects have previously been shown to be clinically mild and transient. Misoprostol does not increase blood pressure and is better tolerated than other oxytocics in terms of nausea, headache and dizziness; however, transient shivering and pyrexia are more common with misoprostol than oxytocin. Reports of misoprostol-induced fevers greater than 40.0°C have been documented following regimens for treating PPH (Hofmeyr et al., 2004; Winikoff et al., 2006). Secondary effects associated with misoprostol, including high fevers, have been transient, easy to manage, non-life threatening, and have not resulted in prolonged hospitalisation. There may also be some minor, but short-lived discomfort from having a blood test. There is a small chance of bruising and an even smaller chance of infection at the site of the blood draw.

A second risk involved in the trial was the possibility that misoprostol is not as effective as oxytocin in treating PPH, meaning that women given misoprostol may have required additional treatment. Based on recent evidence, among women who receive oxytocin during the third stage of labour and go on to experience PPH, treatment with 800 mcg of sublingual misoprostol was similar in effectiveness to IV oxytocin for managing PPH (Blum et al., 2010; Winikoff et al., 2010). To date, however, there is no evidence on how effective a 600 mcg dose of sublingual misoprostol for treatment of PPH will be. All women were under continuous monitoring by a member of the study team. Women who experienced uncontrolled haemorrhages subsequently received the standard of care for treatment of PPH at the study hospital. The hospital has access to all necessary interventions, including gold standard uterotonics, to prevent severe morbidity or mortality resulting from PPH.

The potential benefits to study participants included the possibility that misoprostol will effectively stop excessive postpartum bleeding and reduce the risk of severe

haemorrhage, the need for transfusion, hysterectomy and death. As described above, recent evidence, comparing 800 mcg regimen of sublingual misoprostol to 40 IU IV oxytocin among women receiving oxytocin prophylactically during the third stage of labour, shows that these treatments effectively stop bleeding in 9 out of 10 women. In fact, many dose reduction studies on misoprostol use for other reproductive health indications have demonstrated that lower doses of misoprostol achieved similar results (Carbonell et al., 2008; Khazardoost, Hantoushzadeh & Madani 2007; Sharma, Singhal & Rani 2007). The women had also their blood loss monitored closely to ensure timely treatment for their condition.

The genotypic analysis using the blood samples may also provide us with valuable information that would allow us to develop a way to predict which women are more susceptible to misoprostol-induced side effects and therefore develop better treatment strategies for future patients.

2.2. Liverpool population

107 women were recruited from the Liverpool Women's Hospital termination of pregnancy clinic (Bedford Clinic). The participants had their treatment according to the recommended protocol for TOP in the department (Figure 1).

Sample size calculation

Systematic measurement of elevated body temperature following misoprostol treatment served as the primary outcome measure. Any elevated body temperature measuring greater than or equal to 40.0 °C will be categorized as a 'high fever.' We hypothesize that 15% of women will experience fever following 800 μg regimen of misoprostol for termination of pregnancy, compared to a previously documented rate of 36% following 800 μg in Quito, Ecuador. If we hypothesise that 60% of women who attend Termination of Pregnancy clinic (N=60/week) will agree to participate in our study and that 6 patients/week may experience increased body temperature above 38°C after receiving misoprostol. It is therefore, plausible to plan recruitment of 200 cases within 1 year. To identify these 200 cases and with incidence of 15% of fever in this population, we need to recruit about 1333 women.

2.2.1. Research description and samples collection

All women's temperature was measured before and during treatment at 1, 2 and 4 hours after the treatment. Every woman who developed fever after treatment were considered as a case of misoprostol-induced fever, providing that she was free of these symptoms before taking misoprostol.

The blood sample was obtained by venepuncture and 4–5 ml of blood was collected in a tube containing ethylenediaminetetraacetic acid (EDTA). Then, samples were stored at -20°C freezer in the Bedford Clinic and transferred to the Department of Molecular and Clinical Pharmacology at the University of Liverpool for deoxyribonucleic acid (DNA) extraction and genotyping. The designated people taking the sample were familiar with venepuncture and a septic technique.

2.2.2. Inclusion criteria

The participant women were above 18 years old, had misoprostol for the management of TOP and gave an informed consent (Appendix C. 3. Patient information sheet and consent form).

2.2.3. Exclusion criteria

Any woman younger than 18 years old was not approached by the study researchers for participation in the study for ethical reasons. Also, women with chronic illness and using regular medication were excluded. Women with baseline temperature above 37.0°C and any women who had chills and/or fever during the last 24 hours before admission for TOP were also excluded.

2.2.4. Withdrawal of subjects

Women were free to withdraw from the study at any time should they wish.

2.2.5. Ethical considerations

The study participants were identified by local TOP staff at the Liverpool Women's Hospital and they received detailed written information. All subjects were informed of

the nature and purpose of the study, its requirements and possible hazards, and their right to withdraw at any time from the study without prejudice and without jeopardy to any future medical care at the study site. All subjects had given adequate opportunity to ask the investigator or nominated designee about any aspect of the study. Each participant agreed to co-operate in all aspects of the study and gave informed written consent to the investigator for participation. They were asked to sign three consent forms: one for the investigator, one for the case notes and one for participants. The blood samples were collected from the patient under aseptic technique. Samples for genotypic analysis were coded and stored in a locked freezer in the Department of Molecular and Clinical Pharmacology at the University of Liverpool. These samples were used for the genetic analysis related to this study and will not be used for any other genotypic analysis for any other reasons without the further permission of the Ethics Committee. After collection of clinical data, the coded samples were anonymised, and could not be traceable to any individual subject. Genotypic data was kept by the Molecular and Clinical Pharmacology at the University of Liverpool (under secure conditions) and information will not be routinely disclosed to any third party in any event.

The study was conducted in compliance with the guidelines of the Declaration of Helsinki on biomedical research involving human volunteers (Hong Kong revision, 1989 and the 48th General assembly, Somerset West, Republic of South Africa, October 1996, updated in October 2000) and International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use - Good Clinical Practice guidelines (ICH-GCP guidelines).

Changes to the protocol were made available to the Ethics Committee by means of a formal written protocol amendment and submitted for approval by the Research Ethics Committee. The timing of taking the blood samples had to be changed to the time of consultation rather than the time of admission and to where the women had their blood taken for other necessary investigations as part of their routine care. Therefore, women were not exposed to another needle puncture. This facilitated the recruitment of participants because some women were not happy to have another venepuncture during admission.

2.2.6. Safety Assessment

The safety of the women in this study was of high importance. Participants recruited were not exposed to the procedure of taking blood unless the responsible clinician agreed this would not affect the women's health.

2.3. Data management

All case report forms (CRF) (Appendix C. 4. Case Report Form) were filled out by personnel administering the study procedures in a clear, legible manner. Any changes or corrections were made by drawing a line through the data to be changed, entering the correct information, and signing (or initialling) and dating the change. Every effort was made to have the CRFs completed and signed as soon as possible following blood sample collection. All the study documents were available to the Ethics Committee for inspection on request.

All information obtained as a result of the study is considered confidential and disclosure to any third party other than the Ethics Committee is prohibited. Records identifying the participant were kept confidential.

Documents relating to the study that contained personal data that may have disclosed the identity remained with the investigator in a locked filing cabinet. Participant confidentiality was further assured by anonymising samples at the end of the study and data was not traceable back to any individual patient.

2.4. Outcome measures for the genetic study

Systematic measurement of elevated body temperature following misoprostol treatment is the primary outcome measure. The secondary outcome is the identification of variant genotypes with the highest predictive value for the development of chills and/or fever in women treated with misoprostol.

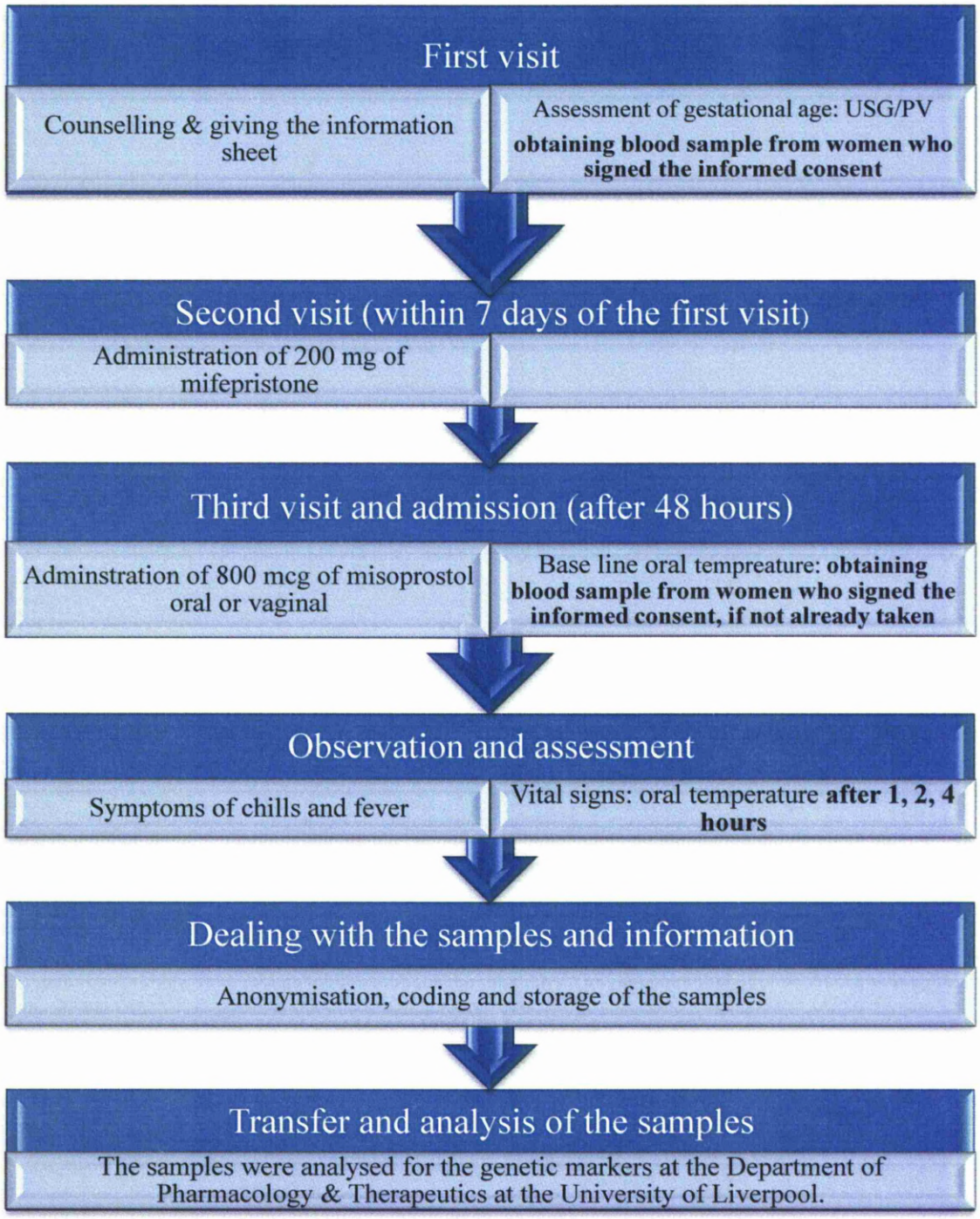

Figure 1. Flow chart for the study procedure incorporated into the Bedford Clinic protocol for termination of pregnancy

3. Materials and Methods

3.1. DNA extraction and quantification

3.1.1. DNA extraction

DNA was extracted from all the samples from Liverpool and Ecuador using Chemagen extraction instrument (PerkinElmer chemagen Technologie GmbH, Germany) (Figure 2). This technology is built around the paramagnetic beads chemistry based on polyvinyl alcohol (M-PVA magnetic beads). The latter is subsequently carboxylated for the easy binding of nucleic acids. Their high magnetite content permits a rapid separation process. The beads have a polydisperse size distribution.

The DNA extraction takes place in two steps. The first step is the lysis step where the protease and the lysis buffer work together to break down the cell and the nuclear membranes to release the DNA into the surrounding solution. The second step is the elution of DNA, where the magnetic beads bind to the DNA helped by the binding buffer. Then, both bind to the magnetic rods and go through several washes in the washing buffers over the racks until the DNA is released in the elution buffer.

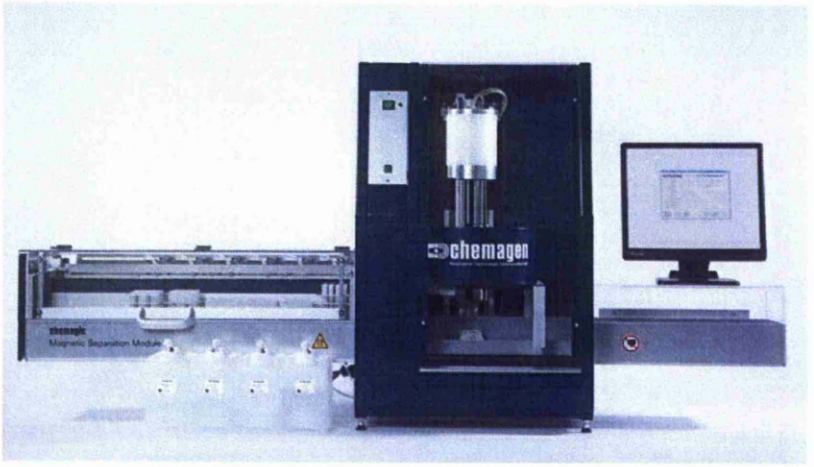

Figure 2. Chemagen extraction machine

4–5 ml blood samples were collected in tubes containing (EDTA) and stored at -20°C in the freezer. The samples were allowed to thaw at room temperature. Chemagen was loaded with rods covers in rack one and empty tubes in racks 2-8. Blood samples, lysis buffer and protease enzyme were added to the tubes in rack 2. The rod mixed the contents and allowed the protease to break down the cell membranes and the nuclear membranes to release the DNA into the surrounding solution. Thereafter, 19.5 ml of binding buffer and 500 µl of magnetic beads were added to the solution. The beads bound to the DNA and then bound to the magnetic rods and were taken through a series of washes through the racks until the DNA was released into the elution buffer in rack 8. Magnetic beads were discarded into rack 7. DNA samples were collected and stored at -4°C for further quantification of the DNA concentration and quality.

3.1.3. DNA quantification

Concentration and quality of the collected DNA samples were measured using the Thermo Scientific NanoDrop 8000 spectrophotometer. This technology is based on the sample retention system which captures the sample between an array of upper and lower optical surfaces. The samples are held in place by the surface tension only and form a column in between the two surfaces. A spectral measurement and quantification of each sample is made based on the tightly controlled path length (Figure 3).

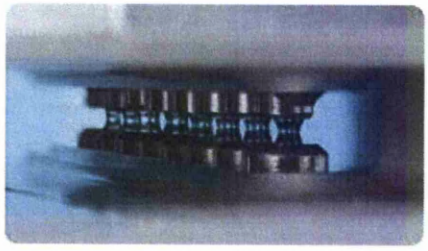

Figure 3. Thermo Scientific NanoDrop 8000 spectrophotometer

After switching on the machine, the water was added to the each pedestal. Then the system was blanked to ensure that the elution buffer in which DNA was suspended did not interfere with the quantification of the samples. Once blanked, 1.5 µl of the samples were added to the appropriate pedestal in the direction A-H.

The result of the DNA quantification displayed as curve represents the absorbance against the wave length as can be seen in Figure 4. The concentration of the DNA is given in ng/µl and the purity of the DNA is given for the quality ratios. These are measures to the purity of the DNA and used to determine if any contamination is present. The ratio of 260/280 around 1.8 and for the ratio 260/230 2 and 2.2 approximately indicated good DNA quality.

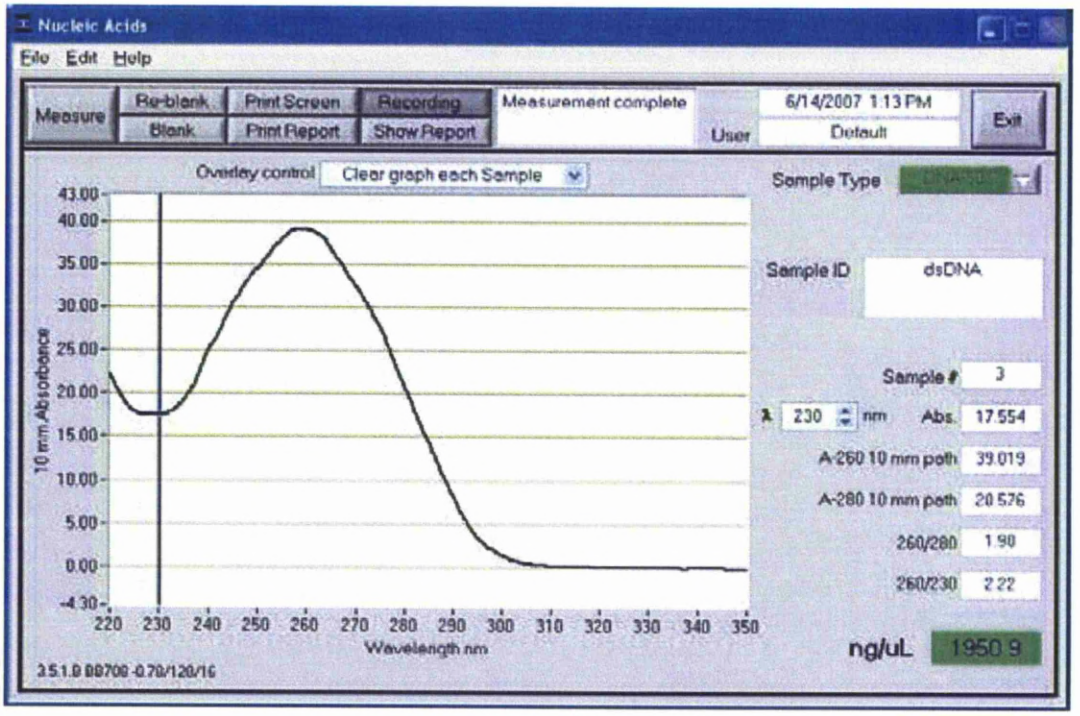

Figure 4. The result of the DNA quantification displayed as a curve represents the absorbance against the wave length

3.2. Candidate gene approach

3.2.1. Bioinformatics gene and SNPs selection

For the genetic analysis, the candidate gene selection was done through searching individual genes and genome-wide association studies (GWAS), from the literature and through the pathway analysis from publically accessible databases, for genes that are involved in the mechanism of action, function and metabolism of misoprostol. 15 genes were explored (Table 1).

Table 1. Candidate genes for misoprostol-induced fever

Chr - chromosome, q- long arm of the chromosome, p- short arm of the chromosome

The candidate genes	Group	Name	Location	Role in fever production
HPGD	Prostaglandin metabolism	15-hydroxyprostaglandin dehydrogenase [NAD+]; NAD+-dependent 15-hydroxyprostaglandin dehydrogenase; prostaglandin dehydrogenase 1; short chain dehydrogenase/reductase family 36C, member 1	Chr4: q34.1 - q34.1	1) Inactivation of PGE_2 catabolising enzymes in other tissues but not in the brain increase the blood-brain gradient of PGE_2 and this mechanism likely facilitates penetration of PGE_2 into the brain and prevents its elimination from the brain (Ivanov, Scheck & Romanovsky 2003).
CR NB, There are CBR 1,3,4	Prostaglandin metabolism	15-hydroxyprostaglandin dehydrogenase; NADPH-dependent carbonyl reductase 1; carbonyl reductase (NADPH); carbonyl reductase (NADPH) 1; carbonyl reductase [NADPH] 1; prostaglandin 9-ketoreductase; prostaglandin-E(2) 9-reductase; short chain dehydrogenase/reductase family 21C, member 1		2) As above (Ivanov, Scheck & Romanovsky 2003).
PGT	Prostaglandin	solute carrier organic anion transporter family,	chr3:	Down regulation of PGT and MOAT in the lung

Alternate symbols: OATP2A1, PGT, **SLCO2A1**	transport	member 2A1 (member of OATP family)	q22.1 - q22.2	and liver but not in the brain would increase blood-brain gradient and likely facilitate penetration of PGE_2 into the CNS and prevent its elimination from the brain (Ivanov, Scheck & Romanovsky 2003). OATP-A strongly expressed in the brain (Tamai et al., 2000)
OATP-C **Alternate symbols: LST-1, LST1, MGC133282, OATP-C, OATP1B1, OATP2, OATPC, SLC21A6** **SLCO1B1**	Prostaglandin transport	solute carrier organic anion transporter family, member 1B1	chr12: p12.2 - p12.1	Expressed at the BBB Expressed mainly in the liver (Tamai et al., 2000).
ABCC4 **Alternate symbols: ABCC4, AF071202.1, EST170205, MOAT-B, MOATB, MRP4**	Prostaglandin transport	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	chr13: q32.1 - q32.1	Down regulation of PGT and MOAT in the lung and liver but not in the brain would increases blood to brain gradient and likely to facilitate penetration of PGE2 into the CNS and prevent its elimination from the brain (Ivanov, Scheck & Romanovsky 2003).
PTGER1 Alternate symbols: EP1	Prostaglandin target	prostaglandin E receptor 1 (subtype EP1), 42kDa	chr19: p13.12 - p13.12	Weak affinity to misoprostol
PTGER2 Alternate symbols: EP2	Prostaglandin target	prostaglandin E receptor 2 (subtype EP2), 53kDa	chr14: q22.1 - q22.1	Clear misoprostol agonist activity
PTGER3	Prostaglandin target	prostaglandin E receptor 3 (subtype EP3	chr1: p31.1 -	Potent and selective misoprostol activity

Alternate symbols: EP3, EP3-I, EP3-II, EP3-III, EP3-IV, EP3e, MGC141828, MGC141829, MGC2730		EP3 (rEP3α & rEP3β)	p31.1	PE3 is 1. Highly expressed in the brain regions such, raphi nuclei (Rpa), hypothalamus preoptic area (POA) and median preoptic nucleus (MnPO) (Nakamura et al., 2002) 2. PGE-EP3 signalling inhibits GABAergic inhibition by changing the $GABA_A$ channels properties. It may affect the sensitivity of these channels to GABA or the response of the POA neurons to GABA (Nakamura et al., 2005). 3. EP3 receptor binds to $G_{i/o}$ proteins in the POA neurons and results in attenuation of *gabr* gene expression (Osaka 2008a, 2008b; Tsuchiya et al., 2008).
PTGER4 **Alternate symbols: EP4, EP4R, MGC126583**	Prostaglandin target	prostaglandin E receptor 4 (subtype EP4)	chr5: p13.1 - p13.1	High affinity to misoprostol PE4 It is mainly expressed in the ventromedial preoptic nucleus (VMPN) and also located in the MnPO (hypothalamus)
GABR (2 genes) **GABRG2,** **GABRA2**	Prostaglandin target	gamma-aminobutyric acid $(GABA)_A$ receptor,	Chr 5, q34 Chr 4, p12	GABA A 1. GABA and $GABA_A$ are essential for PGs to induce febrile response in the POA (Osaka 2008a). 2. PGE-EP3-signalling inhibits GABAergic

				inhibition by rapidly decreasing the expression of gabr gene → ↓ N°_of $GABA_A$ receptors → EP3-expressing neurons become insensitive to the inhibition from GABA → PG induced hyperthermia (Nakamura et al., 2005; Osaka 2008a, 2008b; Tsuchiya et al., 2008).
ADRB1 **Alternate symbols: ADRB1R, B1AR, BETA1AR, RHR**	Prostaglandin target	adrenergic, beta-1-, receptor	chr10: q25.3 - q25.3	BAT heat production (non-shivering thermogenesis) is under sympathetic nervous system control through sympathetic norepinephrine stimulation of B-adrenergic receptors on the brown adipocytes membrane. Inhibition of the B-adrenegic receptor by propranolol or pre-treatment with sympathetic ganglionic blocker chlorisondamine chloride completely prevented the increase in the BAT and core temperature induced by PGE_2 injection to the POAH (Amir & Schiavetto 1990).
ADRB2 **Alternate symbols: ADRB2R, ADRBR, B2AR, BAR, BETA2AR**	Prostaglandin target	adrenergic, beta-2-, receptor, surface	chr5: q32 - q32	As above

ADRB3 **Alternate symbols: BETA3AR, FLJ99960**	Prostaglandin target	adrenergic, beta-3-, receptor	chr8 : p11.23 - p11.23	As above
ADRBK1 **Alternate symbols: BARK1, BETA-ARK1, FLJ16718, GRK2**	Prostaglandin target	adrenergic, beta, receptor kinase 1	chr11 : q13.2 - q13.2	As above

3.2.2. Criteria for the selection of SNPs

After candidate genes have been selected, SNPs selection in candidate genes was performed by searching the literature for reported SNPs, from the SNPs database (dbSNP) and from HapMap. We searched the HapMap for tagging SNPs selection as well. The SNP search within these genes was performed using the SNP database of the NCBI (http://www.ncbi.nlm.nih.gov/SNP).

- The first step of SNPs selection was based on the following criteria:
  Genetic region, types of SNPs and minor alleles frequency (MAF): from SNPs database (dbSNP), we selected all potentially functional SNPs in the exons, 3′ UTR and 5′ UTR regions with minor allele frequency not < (0.05) = 5%. Potentially functional SNPs (missense) are the SNPs which result in production of a different amino acid. A total of 62 potentially functional SNPs in the 15 genes were identified and available for the selection procedure.
- The secondary selection involved the identification of the 62 SNPs in the literature and their association with different diseases or drugs' response. The criteria for selection were identification of SNPs with positive association with a disease or drug response. Accordingly, we identified 13 SNPs from the previously selected 62 SNPs.
- The third selection of SNPs was based on identification of tagging SNPs and linkage disequilibrium. We searched HapMap database (http://www.hapmap.org) for tagging SNPs and we selected SNP pairs with linkage disequilibrium (LD) of 1. Tagging SNPs are representative SNPs in a gene region with high LD (the non-random association of alleles at two or more loci). (For more information, see Chapter 1, section 3). Through these selection criteria, we identified 15 tagger SNPs with high LD.

Combination of the functional SNPs from dbSNP and tagging SNPs from HapMap resulted in a selection of 54 SNPs representing 15 candidate genes (Table 2).

Sequenom assay design was carried out using genotyping tools software from mysequenom website https://www.mysequenom.com/Tools. These tools help to design amplification and extension primers and to design and validate multiplexed

assays. Through this tool we designed 4 plexes. Two of them had 33 SNPs, representing all the candidate genes except PTGER1 (Figure 5). Primers were ordered from Metabion (http://www.metabion.com).

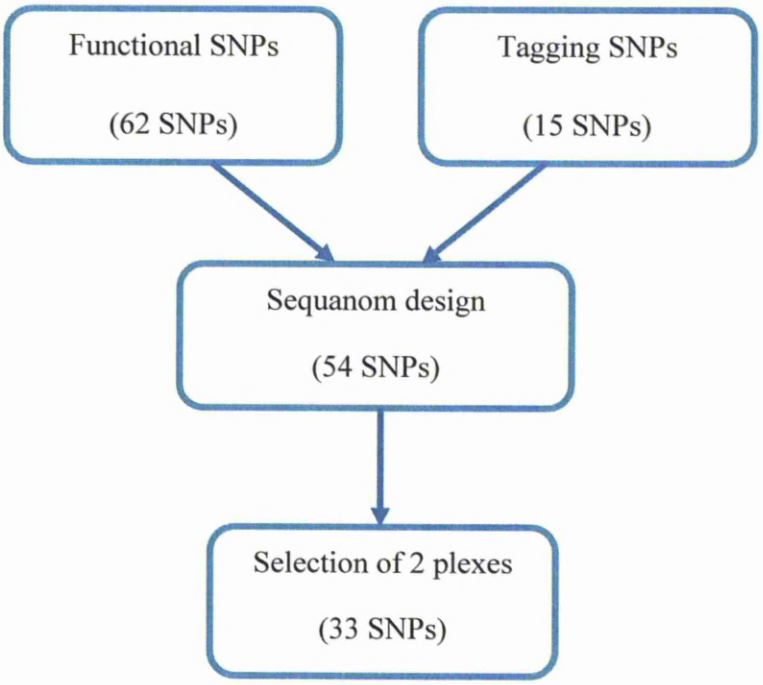

Figure 5. SNPs selection procedure, the combination of the functional SNPs from dbSNP and tagging SNPs from HapMap followed by the sequenom assay design resulted in the final number of SNPs genotyped in our patients (33 SNPs)

Table 2. The selected genes and its functional SNPs, tagging SNPs and examples of their association with some diseases and drugs' response

Gene	SNPs identified from SNPs data base	Studied SNPs Papers/year of publication	Disease studied	SNPs positive association P value	Tagger SNPs/ Linkage disequilibrium
CBR3 (PG metabolism)	rs8133052	(Bains et al., 2010)	Naturally occurring variants of human CBR3 alter Anthracycline in vitro metabolism	+ve P<0.05	rs8133052
	rs16993929				
	rs1056892	(Blanco et al., 2008)	Anthracycline-related congestive heart failure after childhood cancer	-ve	rs879894
HPGD (PG metabolism)	rs45439401				
	rs2555660				
	rs9312555	(Frank et al., 2011)	Colorectal cancer risk	-ve	
	rs8752	(Frank et al., 2011)	Colorectal cancer risk	+ve P=0.009	
	rs2253270				rs2253170
	rs2253170				rs2253170
Prostaglandin transported (OATP2A1) (SLCO2A1)	rs1131597				
	rs2370512				rs6439448
	rs114869610				
	rs72978388				
	rs34550074				
	rs113569514				

OATP-C (SLCO1B1) **Prostaglandin transported**	rs2306283	(Nozawa et al., 2005)	Hepatic uptake of irinotecan	+ve P<0.05	rs2417963
	rs11045819				rs11045825
	rs4149056	(Nozawa et al., 2005) (Takane 2011) (Niemi, Pasanen & Neuvonen 2011)	Hepatic uptake of irinotecan Decreased intake of anti-cancer drug SN-38 from the systemic circulation, leading to an increase in its plasma concentration and enhancing the risk of neutropenia Associated with statin-induced adverse drug reactions	+ve P<0.05 +ve P<0.05 +ve P<0.05	rs11045879
	rs34671512				
	rs4149085				
	rs4149087				rs4149087
	rs11045891				rs11045825
	rs4149088				rs4149087
ABCC4/MRP 4 **Prostaglandin transporter**	rs9590161				
	rs34559063				
	rs9516519				
	rs16950472				
	rs9516520				
	rs1059751				rs4148553
	rs9516521				
	rs4148553				rs4148553

	rs4148551				
	rs3742106	(Rodríguez-Nóvoa et al., 2009)	Kidney tubular necrosis in HIV patients treated with tenofovir	-ve	rs3742106
	rs3765534	(Ban et al., 2010) (Krishnamurthy et al., 2008)	Thiopurine sensitivity in Japanese patients with inflammatory bowel disease Transporter-mediated protection against thiopurine-induced hematopoietic toxicity	+ve P=0.036 +ve P<0.05	rs3765534
	rs2274407	(Izzedine et al., 2006)	No Association between *ABCC4* Gene Haplotypes and Tenofovir-induced proximal tubulopathy	-ve	
PTGER1	rs7249305				
	rs28364035				
PTGER2 (prostaglandin target)	rs1353411	(Park et al., 2007) (Kim et al., 2007)	Asthma in the Korean population Aspirin-intolerant asthma	+ve P=0.002 -ve	
	rs1254598	(Park et al., 2007)	Asthma in the Korean population	+ve P=0.043	
	rs708502	(Park et al., 2007)	Asthma in the Korean population	+ve P<0.05	

	rs17197	(Sato et al., 2007)	Essential hypertension in all but in men	-ve +ve (p=0.041)	rs17197
	rs45461592				
	rs1042618	(Park et al., 2007)	Asthma in the Korean population	-ve	rs17197
PTGER3 (prostaglandin target)	rs5697				rs3819783
PTGER4 (prostaglandin target)	rs16870224	(Kim et al., 2007)	Aspirin-intolerant asthma	-ve	rs16870224
GABRG2 (prostaglandin target)	rs211035				
	rs418210				
	rs424740	(Pham et al., 2009)	Anxiety spectrum disorders	-ve	
GABRA2 (prostaglandin target)	rs573400	(Agrawal et al., 2008) (Haughey et al., 2008) (Agrawal et al., 2006)	Nicotine dependence Acute effects of alcohol Alcohol dependence and drug dependence	+ve P=0.008 +ve P<0.05 +ve P=0.009	rs279860
	rs76026776				
ADRB1 (prostaglandin target)	rs1801252	(Leu et al., 2011) (Bengtsson et al., 2010) (Leineweber et	Carotid intima-medial thickness Hypertension, and Obstructive Sleep Apnoea Exercise capacity and survival in	-ve +ve P=0.042 -ve	

		al., 2010)	patients with end-stage heart failure		
		(Nia et al., 2010)	Predicts Flecainide action in patients with atrial fibrillation	+ve P=0.03	
		(Nicoulina et al., 2010)	Association with atrial fibrillation	+ve P<0.05	
	rs1801253	(Johnson et al., 2011)	Hypertension	+ve $P=4.7x10^{-10}$	rs1801253
		(Petersen et al., 2011)	Mortality in Carvedilol-treated chronic heart-failure patients	+ve RR=2.3	
		(Bengtsson et al., 2010)	Hypertension, and obstructive sleep Apnoea	-ve	
		(Leineweber et al., 2010)	Exercise capacity and survival in patients with end-stage heart failure	-ve	
		(Hakalahti et al., 2010)	Left ventricular hypertrophy	+ve P=0.001	
		(Baudhuin et al., 2010)	Response to, Carvedilol or Metoprolol therapy in Patients With chronic heart failure	+ve P=0.01	
		(Nia et al., 2010)	Predicts Flecainide action in patients with atrial fibrillation	+ve P=0.03	

		(Poon et al., 2010)	Cardiovascular disease in patients with diabetic nephropathy	-ve	
ADRB2 (prostaglandin target)	rs1801704	----------			rs1801704
	rs1042711	----------			
	rs1042713	(Leineweber et al., 2010)	Exercise capacity and survival in patients with end-stage heart failure	-ve	rs1042713
		(Pereira, Mingroni-Netto & Yamada 2011)	Risk of obesity in Japanese	-ve	
		(Schürks et al., 2009)	Migraine in women	-ve	
	rs1042714	(Petersen et al., 2011)	Mortality in Carvedilol-treated chronic heart-failure patients	-ve	
		(Leu et al., 2011)	Carotid intima-medial thickness	-ve	
		(Leineweber et al., 2010)	Exercise capacity and survival in patients with end-stage heart failure	-ve	
		(Schürks et al., 2009)	Migraine in women	-ve	
ADRB3 (prostaglandin target)	rs34434657				
	rs35646917				
	rs4999				
	rs4998				

	rs4994	(Wang et al., 2011) (Tahara, Osaki & Kishimoto 2010) (Yamakita et al., 2010) (Wang et al., 2010) (Cruz et al., 2010) (Fan et al., 2010) (Morcillo et al., 2010) (Marvelle et al., 2008) (Dunajska et al., 2008)	Gout in Chinese male population BMI reduction Weight changes in obese Japanese Men Cardiac disease in type 2 diabetes Type 2 diabetes and metabolic syndrome from Mexico City Cardiovascular risk markers Hyperuricemia risk in a population from southern Spain BMI and obesity Metabolic syndrome in postmenopausal women	+ve P=0.027 -ve +ve P=0.002 +ve P=0.002 +ve P=0.001 +ve P<0.05 +ve P=0.017 +ve P<0.05 -ve	rs4994
ADRBK1 **(prostaglandin target)**	rs114509093				
	rs4370946				

3.3. Method for SNPs genotyping

3.3.1. Principle of SNPs genotyping using the Sequenom Mass ARRAY iPLEX Platform

The Sequenom Mass ARRAY system (Figure 6) is based on the technology of Matrix Assisted Laser Desorption/Ionisation-Time-of-Flight Mass Spectrometry (MALDI-TOF MS). Mass spectrometry is used to identify the SNP alleles based on the distinct mass of the extended primer. The primer's mass indicates the sequence and, consequently, the alleles present at the polymorphic site of interest. The Sequenom (Spectro Typer) software automatically translates the mass of the observed primers into a genotype for each reaction.

Figure 6. Matrix Assisted Laser Desorption/Ionisation-Time-of- Flight Mass Spectrometry (MALDI-TOF MS)

Brief description of the process of Mass ARRAY genotyping

This section briefly describes the process of Mass ARRAY genotyping and for detailed description refers to the Protocol (Appendix C.5). Figure 7 shows a summary of the process of genotyping using MassARRAY system (MALDI-TOF MS).

Prior to the procedure, the DNA samples are diluted to 20 ng/ml using 1xTE (Tris-EDTA) buffer. The DNA samples need to be amplified by PCR prior to performing the extension reaction of the SNPs of interest.

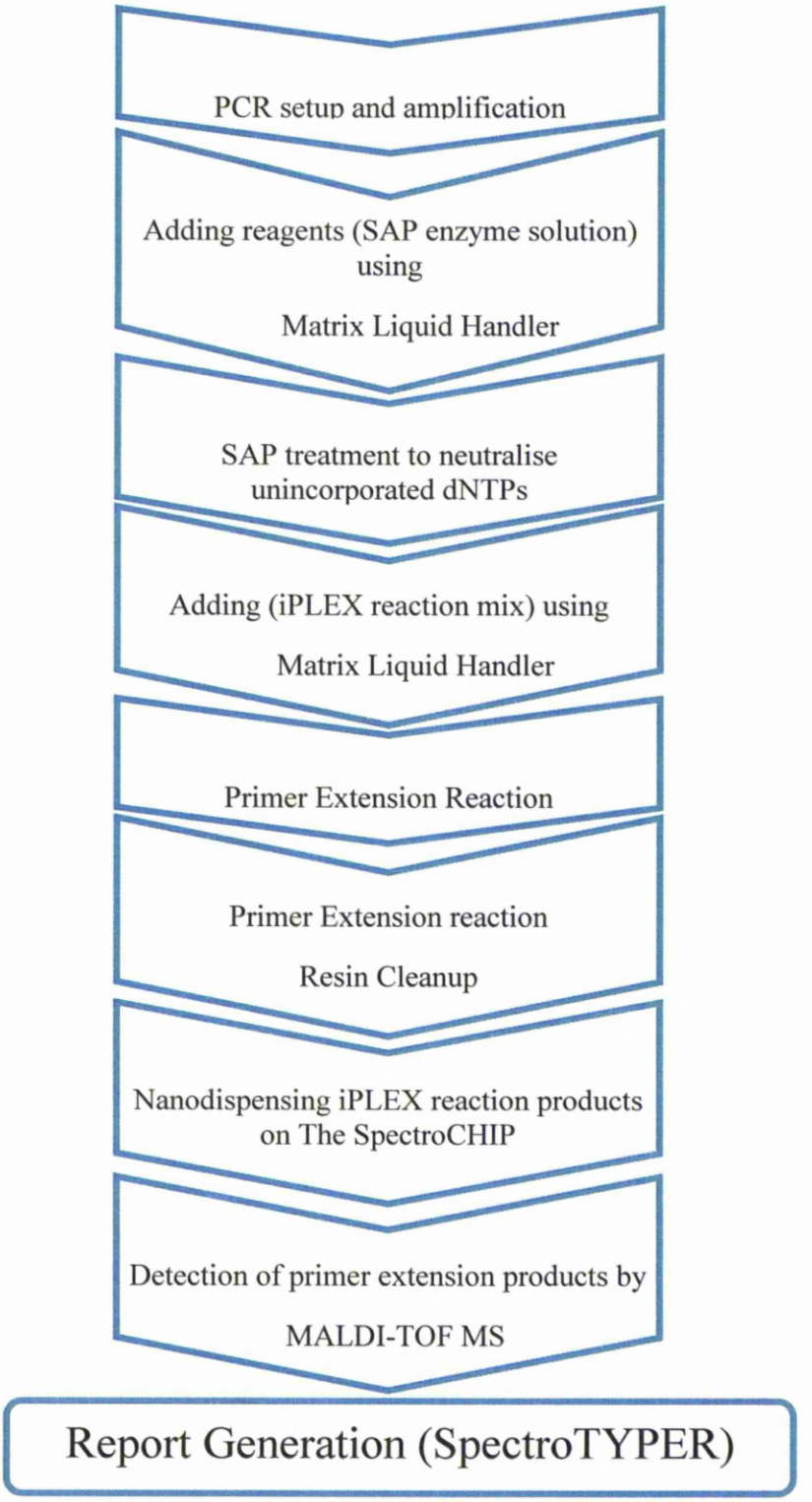

Figure 7. Process of Genotyping using Mass ARRAY system (MALDI-TOF MS)

PCR setup and amplification

This step includes amplifying a selected fragment of genomic DNA for genotyping on the Mass ARRAY platform. The goal of multiplex PCR is to amplify many loci of DNA with minimal nonspecific by-products. The purified amplicons are used as templates for the primer extension reaction.

The PCR mix, which contains PCR buffer, $MgCl_2$, dNTP mix, primer mix, and Hot Star Taq, undergoes thermal cycling (PCR reaction). Multiplexed assays for the master mix (PCR primers, extension primers) as shown in Appendix C. 6.

SAP reaction cleanup

Treatment with shrimp alkaline phosphatase (SAP) is performed to remove the remaining, non-incorporated dNTPs from amplification products. This takes place by cleaving the phosphate groups from the 5′ termini of the non-incorporated dNTPs. This procedure is performed by using the Matrix Liquid Handler robot. Incubation of the reaction is at 37°C for 40 minutes and is then followed by incubation at 86°C for 20 minutes to inactivate SAP enzyme on thermal cycler.

Primer Extension Reaction

Primer Extension Reaction or iPLEX Reaction is a method for detecting single base polymorphisms or small insertion/deletion polymorphisms in amplified DNA. The primer extension reaction cocktail which contains iPLEX buffer, iPLEX-Termination mix, Primer mix and iPLEX enzyme are added to the amplification products. The addition of the iPLEX reaction is performed using Matrix Liquid Handler. During the iPLEX reaction, the primer is extended by one mass-modified dideoxynucleotide terminator of an oligonucleotide primer which anneals immediately upstream of the polymorphic site of interest. The primer extension is performed by thermal cycling.

Primer Extension Reaction resin cleanup

The cleanup step is essential to optimise mass spectrometry analysis of the extended reaction products. The cationic resin helps to remove salts such as Na^+, K^+ and Mg^{2+}

ions. The presence of these ions can result in high background noise in the mass spectra.

Nanodispensing iPLEX reaction products on the SpectroCHIP

The extended/desalted iPLEX reaction products are dispensed from 384-well microtiter plate on 384-sample SpectroCHIP. A small volume of the iPLEX products (25 nl) is arrayed onto the existing matrix spots on the silica chip and this allows for precise incorporation of oligonucleotides with the appropriate matrix for MALDI-TOF.

Detection of primer extension products by mass spectrometry

The extended primer products are detected using Mass ARRAY spectrometer and Sequenom real-time detection software.

In the mass spectrometer, once the laser beam is fired at the chip, the matrix made of crystallised molecules is ionised and part of its charge is transferred to the analyte molecules, which become ionised as well. The matrix molecules have several roles: 1) to absorb most of the laser energy, 2) to protect the analyte molecules from being destroyed by direct laser and 3) to prevent ion fragmentation (soft ionisation). The rapidly expanding matrix plume carries some of the analyte into the vacuum with it and this aids the sample ionisation process. The vaporised and ionised sample molecules are transferred electrostatically into time-of-flight mass spectrometer where they are separated from the matrix ions and individually detected based on their mass to charge ratio *(m/z)* and analysed by the software. Ion detection at the end of the tube is based on its flight time as molecules with large mass transfer slower than molecules with small mass. Flight time is proportional to the square root of its *m/z*. Therefore, the unique mass values of the extended primer allow discrimination between two alleles and identification of the SNPs (Figure 8).

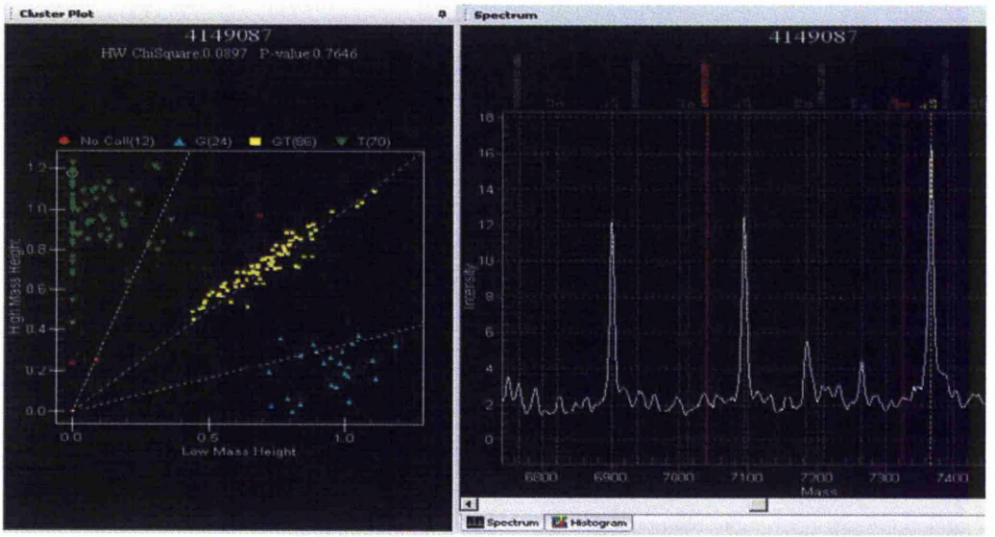

Figure 8. Cluster plot of low mass allele vs. high mass allele for chosen assay

Spectrum of a chosen assay shows analyte signals, genotypes and mass range

3.4. Statistical analysis

Statistical analysis of incidence means ±SD were performed by Excel. Continuous data analysis in respect to genotypes and its association with body temperature in women on misoprostol (phenotype) was assessed using analysis of variance (ANOVA) with correction of multiple comparisons using Benfrroni method in SPSS statistics 19. The difference was taken as statistically significant when P values were less than 0.05.

4. Results

4.1. Clinical data result

4.1.2. Ecuador population

4.1.2.1. Characteristics of the study Ecuadorian population

766 women were enrolled in the study and screened for the diagnosis of PPH. 50 women were included who were treated for PPH with 600 mcg sublingual misoprostol. Table 3 shows the characteristics of the included population.

Table 3. Mean of women's age, number of live births and BMI in the Ecuadorian population

Characteristics	Mean ± SD
Age	23.08 ± 0.84
Number of live births	1.72 ± 0.14
BMI	24.5 ± 0.5

4.1.2.2. Ethnicity of the included Ecuadorian population

The study population included 5 different ethnic groups from Ecuador. The most common ethnic group was Mestizo, which means mixed population from indigenous and Europeans. This group represents 44 women with incidence of 88% of the included population (Table 4).

Table 4. Incidence of different ethnic groups in the Ecuadorian population (Mestizo: Indigenous and European) (Mulato: mixed white and black)

Ethnicity	Number	Percentage (%)
Indigenous	1	2.0
Black	1	2.0
Mestizo	44	88.0
Mulato	2	4.0
White	2	4.0

4.1.2.3. Frequency and incidence of side effects after treatment with 600 mcg sublingual misoprostol in Ecuadorian population

As illustrated in Table 5, the commonest side effect was chills or shivering with an incidence of 96%, followed by the fever, which occurred in 90 % of the population.

Table 5. Frequency and incidence of different side effects after treatment of Ecuadorian women with 600 mcg sublingual misoprostol for PPH

Side effects	Number	Incidence (%)
Nausea	0	0
Vomiting	0	0
Diarrhoea	0	0
Chills/Shivering	48	96
Fever $\geq$ 37.6 °C	46	90
Fainting	2	4
Others Headache	1	2

4.1.2.4. Incidence of different categories of body temperature in the Ecuadorian population

Body temperature measurements from the 50 women who were treated for PPH were classified to 4 categories. Body temperature < 37.6°C occurred in 4 women, which represents 8% of the population under study; body temperature from 37.6°C to 39°C was reported in 33 (66%) of the population; body temperature of 39.1°C to 39.9°C was observed in 8 (16%) of the treated women; and body temperature >40°C was recorded in 5 (10%) of the population (Figure 9)

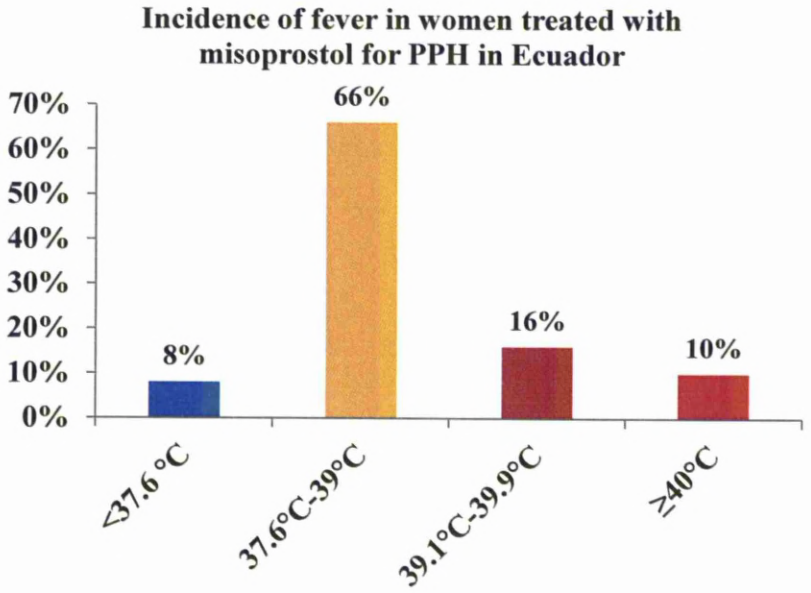

Figure 9. Incidence of different categories of fever in Ecuadorian women treated with misoprostol for PPH

4.1.2.5. Number of women in different fever categories in different ethnic groups Ecuadorian population

The commonest fever category was the second (37.6°C–39°C), which represents about 72% of the participant women with fever (46 women), followed by the third category (39.1°C–39.9°C), which accounts for 17.4% of the febrile women. All women with high fever > 40°C were from the mestizo population and they represent 11.9% of the febrile women population (Table 6).

Table 6. Number of women in different fever categories in Ecuador population according to the ethnic groups

Fever Category	Indigenous	Black	Mestizo	Mulato	white	Total
<37.6 °C (No fever)	3		1			4
37.6°C–39°C	1		29	1	2	33/46 72%
39.1°C–39.9°C			7	1		8/46 17.4%
> 40 °C			5			5/46 10.8%

4.2.2. Liverpool population

4.2.2.1. Characteristics of Liverpool population

107 women who were seeking (TOP) were recruited for the study and they received 800 mcg vaginal misoprostol. 93 of the recruited women had complete data about fever and had their blood samples collected for the genetic analysis. Therefore, we included only these women in the statistical analysis of data. Table 7 shows the characteristics of the included population.

Table 7. Means and SD for gravidity, parity and BMI of the Liverpool population

Characteristics	Mean ± SD
Gravidity	2.5 ± 0.2
Parity	0.9 ± 0.1
BMI	27.5± 1.2

4.2.2.2. Ethnicity of the included Liverpool population

The study population included 7 different ethnic groups. The commonest ethnic group was White British and it represented about 85% of the total population. Other ethnic groups were very rare with not more than 3 individuals in each group (Table 8).

Table 8. Incidence of the different ethnic groups in Liverpool population

Ethnicity	Number	Percentage (%)
White	87	92.3
Black	2	1.1
Other Ethnic Background	1	1.1
Other Mixed Background	2	2.1
Indian	1	1.1

4.2.2.3. Frequency and incidence of side effects after treatment with 800 mcg vaginal misoprostol in Liverpool population

The commonest side effect was abdominal cramps (77.7%), followed by feeling of coldness (55.3%), whereas fever is the least common side effect and occurred in 8.8% of the treated women. The figures for other side effects are illustrated in Table 9.

Table 9. Incidence of side effects of misoprostol in the participant women from Liverpool

Side effects	Number	Incidence (%)
Coldness	52	55.3
Chills and/or shivering	30	31.9
Fever ≥ 37.6 °C	8	8.8
Nausea and vomiting	36	38.3
Diarrhoea	19	20.2

4.2.2.4. Incidence of different categories of body temperature in Liverpool population

Body temperature measurements from the 91 women who underwent TOP were classified into 3 categories. Body temperature ≥ 37.6°C was considered a diagnosis of fever and less than this figure was considered as no fever. Women with body temperature ≥ 37.6°C represented around 9% of the total population (Figure 10).

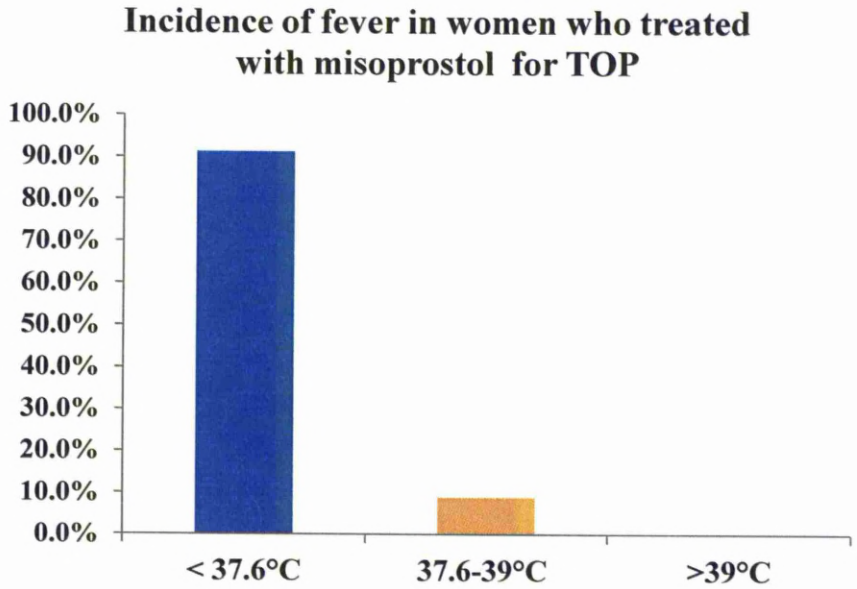

Figure 10. Incidence of different categories of body temperature in Liverpool population

4.2.2.5. Incidence of fever according to ethnicity

All women who were diagnosed with fever (body temperature ≥ 37.6°C) were White. None of the other ethnic groups had fever.

4.2. Results of the SNPs genotyping

Genotyping quality control

Genotyping was performed in 143 subjects (50 Ecuadorian and 93 Liverpool women) for 33 known SNPs in 14 genes. Our overall genotyping sample success rate (call rate) was 90%. All the SNPs were genotyped and included in the final data analysis.

Call rate= Precent of gynotypes with calls other than low propability or no call out of the total number of possible calls

4.2.1. Ecuador population

4.2.1.1. The association between genotypes and body temperature

The genotype and phenotype data were used to determine if there was any association between the mean temperature and its genotype. There was an association between 2 SNPs (rs4149085 and rs4149087) in the prostaglandin transporter (SLCO1B1) (P=0.027 and P=0.005) respectively. Also, there was an association between the mean temperature and SNP (rs114869610) in prostaglandin transporter (SLCO2A1) with P=0.017 (Table 10 and Figures 11, 12 & 13).

Table 10. Allelic frequencies and genotype frequencies for the selected SNPs in 14 genes and their mean temperature in Ecuadorian women treated with misoprostol for treatment of PPH

Gene	dbSNP	Location	Allele	Allelic Frequency	Genotype	Count (%)	mean temperature (°C) (SD)	P value	NCBI /MAF
SLCO1B1	rs11045825	Chr12	T	0.97	TT	47 (94)	38.8 (±0.8)	0.23	0.086
		Intron	C	0.03	CT	3 (6)	38.2 (±0.9)		
	rs11045879	Chr12 Intron	C	0.195	CC	1 (2)	38.7 (0)	0.643	0.26
			T	0.805	TC	17 (35)	38.97 (±1.0)		
					TT	31 (63)	38.7 (±0.8)		
	rs4149085	Chr12	C	0.01	CT	1 (2)	40.7 (0)	0.027	0.123
		3' UTR	T	0.99	TT	47 (98)	38.74 (±0.8)		
	rs4149087	Chr12	G	0.35	GG	7 (14)	39.6 (±0.9)	0.005	0.48
		3' UTR	T	0.65	GT	21 (42)	38.4 (±0.7)		
					TT	22 (44)	38.8 (±0.8)		
	rs34671512	Chr12	A	0.99	AA	48 (98)	38.8 (±0.9)	0.367	0.56
		missense	C	0.01	CA	1 (2)	38.0 (0)		
SLCO2A1/ OATP2A1	rs34550074	Chr3	A	0.306	AA	6 (14.6)	38.8 (±0.9)	0.5	0.28
		missense	G	0.694	GA	13 (32)	39.0 (±0.9)		
					GG	22 (53.4)	38.6 (±0.8)		
	rs113569514	Chr3	C	0.265	CC	4 (11)	38.47 (±0.9)	0.756	0.33
		5' UTR	T	0.735	CT	11 (31)	38.8 (±0.9)		
					TT	21 (58)	38.8 (±0.8)		
	rs6439448	Ch43	A	0.342	AA	6 (13)	39.4 (±1.0)	0.08	0.25
		intron	C	0.658	CA	20 (42.5)	38.57(±0.8)		
					CC	21 (44.5)	38.75(±0.7)		
	rs72978388	Chr3	T	0.99	TT	49 (98)	38.8 (±0.8)	0.135	0.052

		3' UTR	A	0.01	TA	1 (2)	37.5 (0)		
	rs114869610	Chr3	A	0.99	AA	45 (98)	38.75 (±0.8)	**0.017**	0.07
		3' UTR	C	0.01	CA	1 (2)	40.7 (0)		
ABCC4/ MRP4	rs2274407	Chr13	A	0.073	AA	1 (2.1)	38.7 (0)	0.99	0.16
		missense	C	0.916	CA	5 (10.4)	38.8 (±0.6)		
			T	0.011	TC	1 (2.1)	39 (0)		
					CC	40 (85.4)	38.8 (±0.9)		
ABBCC4/ MR	rs3742106	Chr13	A	0.48	AA	12 (25)	38.9 (±0.7)	0.86	0.39
		3' UTR	C	0.52	CA	22 (46)	38.76 (±0.9)		
					CC	14 (29)	38.76 (±0.9)		
	rs4148551	Chr13	A	0.48	AA	12 (25)	38.9 (±0.7)	0.864	0.48
		3' UTR	G	0.52	GA	22 (46)	38.7 (±0.9)		
					GG	14 (29)	38.7 (±0.8)		
	rs34559063	Chr13	A	0.53	AA	16 (34.7)	38.66 (±0.9)	0.51	0.424
		3' UTR	G	0.47	GA	17 (37)	38.88(±0.8)		
					GG	13 (28.3)	39.04 (±0.8)		
	rs3765534	Chr13	C	0.95	CC	42 (88)	38.67 (±0.8)	0.24	0.061
		missense	T	0.05	CT	5 (10)	39.18 (±1.0)		
					TT	1 (2)	39.7 (0)		
	rs4148553	Chr13	A	0.27	AA	4 (10.3)	39.7 (±1.0)	0.065	0.42
		3' UTR	G	0.73	AG	13 (33.3)	38.8 (±0.6)		
					GG	22 (56.4)	38.6 (±0.9)		
PTGER2	rs17197	Chr14	C	0.235	CC	4 (8)	38.97 (±0.4)	0.91	0.28
		3' UTR	T	0.765	TC	15 (31)	38.75 (±0.8)		
					TT	30 (61)	38.79 (±0.9)		
	rs708502		A	0.25	AA	5 (10)	38.8 (±0.4)	0.96	0.28
			G	0.75	AG	15 (30)	38.7 (±0.8)		
					GG	29 (60)	38.7 (±0.9)		

	rs45461592	Chr14 3' UTR	A G	0.97 0.03	AA TA	45 (94) 3 (6)	38.8 (±0.8) 38.2 (±0.5)	0.285	0.05
PTGER3	rs3819783	Chr1 Intron	C T	0.255 0.745	CC CT TT	4 (8.9) 15 (33.3) 26 (57.8)	39.025 (±1.0) 38.62 (±0.7) 38.86 (±0.9)	0.646	0.244
PTGER4	rs16870224	Chr5 3' UTR	A G	0.12 0.88	AG GG	11 (24) 34 (76)	38.77 (±0.9) 38.87 (±0.8)	0.74	0.133
ADRB1	rs1801253	Chr10 missense	C G	0.97 0.03	CC GG	31 (97) 1 (3)	38.65 (±0.9) 37.9 (0)	0.415	0.294
ADRB2	rs1042714	Chr5 missense	C G	0.965 0.035	CC GC	42 (93) 3 (7)	38.87 (±0.9) 38.23 (±1.2)	0.228	0.24
ADRB3	rs4998	Chr8 3' UTR	C G	0.02 0.98	CG GG	2 (4) 47 (96)	38.2 (±0.4) 38.8 (±0.9)	0.34	0.095
GABRG2	rs211014	Chr5 Intron	A C	0.417 0.583	AA CA CC	3 (8.3) 24 (66.7) 9 (25)	39.1 (±0.87) 38.8 (±0.96) 38.9 (±0.59)	0.866	0.38
	rs211035	Chr5 missense	A G	0.07 0.93	AG GG	6 (14.3) 36 (85.7)	39.06 (±0.9) 38.65 (±0.8)	0.259	0.20
HPGD	rs2253170		A G	0.065 0.935	AA AG GG	1 (3.2) 2 (6.5) 28 (90.3)	37.5 (0) 39.9 (±1.34)	0.12	0.05
	Rs45439401		C T	0.315 0.685	CC TC TT	7 (16) 14 (31) 24 (53)	39.16 (±0.7) 38.6 (±0.8) 38.7 (±0.9)	0.4	
CBR3	rs879894	Chr21 Intron	A C	0.177 0.823	AA CA CC	3 (6.2) 11 (23) 34 (70.8)	38.96 (±0.8) 38.86 (±0.7) 38.77 (±0.9)	0.9	0.4

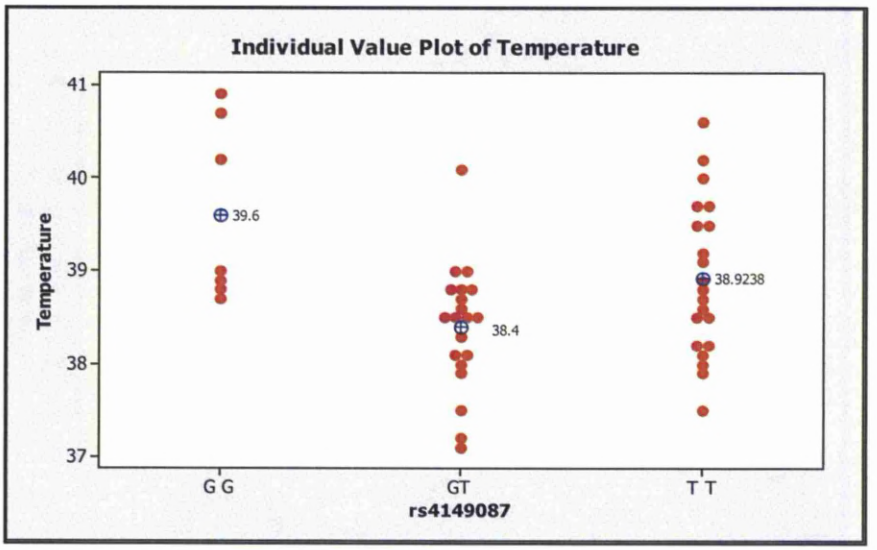

Figure 11. Individual plot of temperature showing the mean temperature in each genotype for rs4149087 in the prostaglandin transporter gene SLCO1B1 (P=0.005)

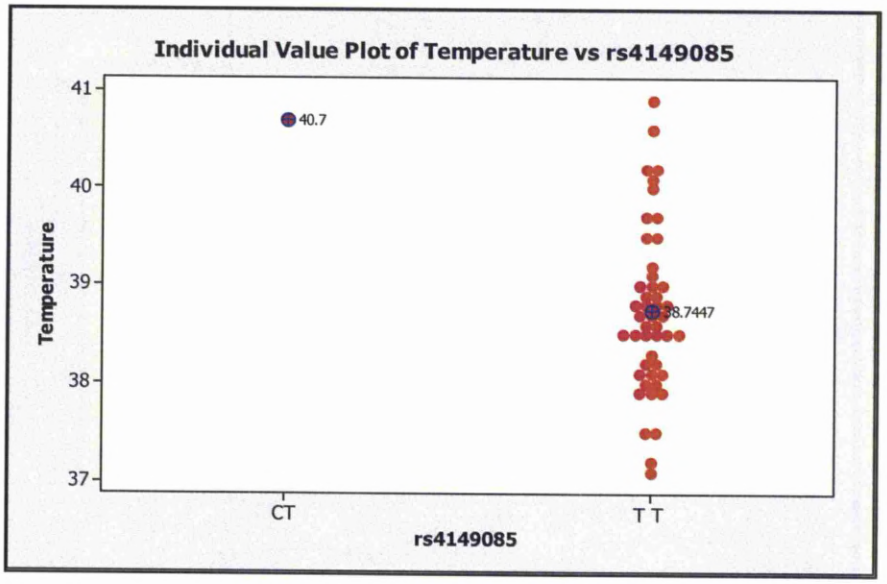

Figure 12. Individual plot of temperature showing the mean temperature in each genotype for rs4149085 in the prostaglandin transporter gene SLCO1B1 (P=0.027)

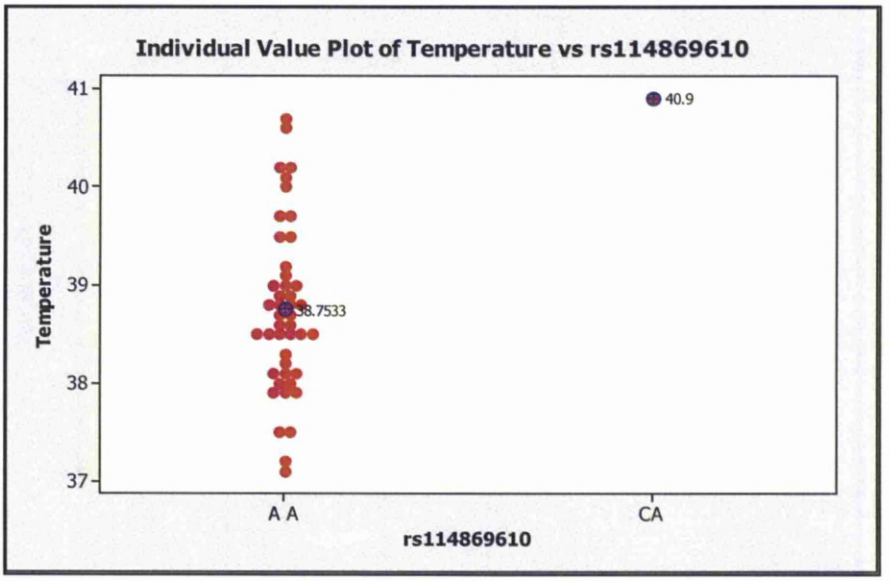

Figure 13. Individual plot of temperature showing the mean temperature in each genotype for rs114869610 in the prostaglandin transporter gene SLCO2A1 (P=0.017)

4.2.1.2. Comparison of Ecuadorian population with other Hispanic/Latino populations

As can be seen in Figure 14 from a genome-wide patterns study of Hispanic/Latino population, the Ecuadorian population (light green) showed similar genetic profile to Colombian (yellow) and Mexican (light blue) populations. The comparison of minor allele frequency (MAF) of these populations with this study Ecuadorian population is shown in Table 11. The MAF is actually the second most frequent allele value.

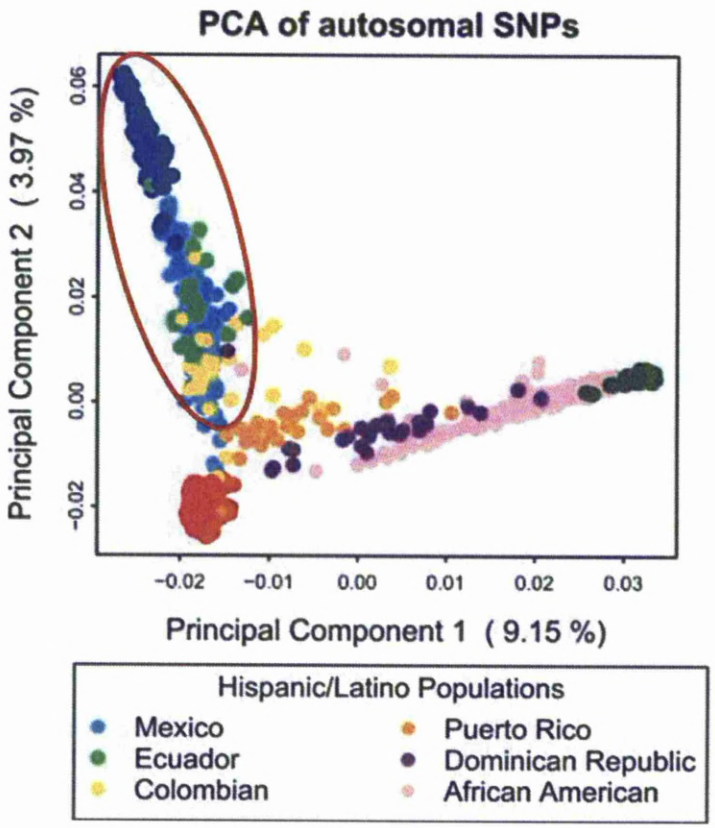

Figure 14. Principal component analysis results of the Hispanic/Latino individuals. Adapted from Bryc et al. 2010

Table 11. Allelic frequency of SNPs with positive association with misoprostol-induced fever (rs4149087) in this study Ecuadorian population and in twenty Ecuadorian and twenty-six Colombian populations (healthy volunteers) from a study of genome-wide patterns of population structure and admixture among Hispanic/Latino populations (Bryc et al. 2010)

Population (Number)	MAF rs4149087
Ecuadorian (20) **(Bryc et al., 2010)**	G=0.4
Colombian (26) **(Bryc et al., 2010)**	G=0.36
Our study population (Ecuador) (50)	G=0.35
Caucasian (North America) (NCBI)	G=0.36
MAF/global NCBI	G=0.47

4.2.2. Liverpool population

4.2.2.1. The association between genotypes and body temperature

The genotype and phenotype data was used to determine if there was any association between the mean temperature and its genotype. There was no genetic association with mean temperature in the Liverpool population treated with misoprostol for TOP (Table 12).

Table 12. Allelic frequencies and genotype frequencies for the selected SNPs in 14 genes and their mean temperature in Liverpool women treated with misoprostol for TOP

Gene	dbSNP ID	Location	Allele	Frequency (%)	Genotype	Frequency N (%)	Mean temperature (°C) (SD)	P value	NCBI / MAF (%)
SLCO1B1	rs11045825	Chr12 Intron	C T	15.5 84.5	CC CT TT	1 (1) 26 (29) 70 (63)	36.4 (0) 36.7 (±0.8) 36.9 (±0.5)	0.24	8.6
	rs11045879	Chr12 Intron	C T	16 84	CC TT TC	1 (1.2) 58 (69) 25 (29.8)	36.0 (0) 36.84 (±0.6) 36.8 (±0.6)	0.86	26
	rs4149085	Chr12 3' UTR	C T	0.5 99.5	CT TT	1 (1) 84 (99)	37.3 (0) 36.8 (±0.6)	0.4	12.3
	rs4149087	Chr12 3' UTR	G T	40 60	GG GT TT	14 (16.3) 41 (47.7) 31 (36)	36.7 (±0.7) 36.8 (±0.6) 36.9 (±0.5)	0.5	48
	rs34671512	Chr12 Missense	A C	93.5 6.5	AA CA	80 (87) 12 (13)	36.8 (±0.7) 37.0 (±0.5)	0.5	5.6
SLCO2A1 /OATP2A1	rs34550074	Chr3 Missense	A G	13.8 86.2	AA GG GA	4 (5.5) 57 (78) 12 (16.5)	36.6 (±0.5) 36.9 (±0.5) 36.8 (±0.7)	0.6	28

	rs114869610	Chr3 3' UTR	A C	97 3	AA CA	82 (94.3) 5 (5.7)	36.8 (±0.6) 37.2 (±0.18)	0.16	?
	rs113569514	Chr3 5' UTR	C T	18 82	CC CT TT	5 (6) 20 (24) 59 (70)	36.7 (±0.5) 36.8 (±0.8) 36.9 (±0.6)	0.4	33
	rs6439448	Chr3 Intron	A C	76.4 80.6	AA CC CA	5 (5.4) 61 (66.3) 26 (28.3)	36.7 (±0.6) 36.9 (±0.6) 36.8 (±0.7)	0.5	0.25
	rs72978388	Chr3 3' UTR	T A	99 1	TT TA	91 (98) 2 (2)	36.9 (±0.6) 37.3 (±0.3)	0.4	5.2
ABCC4/ MRP4	rs2274407	Chr13 Missense	A C T	6.7 89.3 4	AA CC AC TC	1 (1.4) 60 (80) 8 (10.6) 6 (8)	37.2 (0) 36.8 (±0.6) 37.0 (±0.9) 36.7 (±0.7)	0.7	16
	rs3742106	Chr13 3' UTR	A C	51.5 48.5	AA CC CA	26 (30) 24 (27) 38 (43)	36.8 (±0.5) 37.0 (±0.5) 36.7 (±0.7)	0.1	39
	rs4148551	Chr13 3' UTR	A G	50 50	AA GG GA	25 (30) 25 (30) 35 (40)	36.8 (±0.5) 37.1 (±0.5) 36.7 (±0.7)	0.09	48
	rs34559063	Chr13 3' UTR	A G	40.5 59.5	AA GG GA	25 (27) 42 (46) 25 (27)	37.1 (±0.5) 36.7 (±0.5) 36.8 (±0.8)	0.3	42.4
	rs3765534	Chr13 missense	C T	1.5 98.5	CC CT	90 (97) 2 (3)	36.8 (±0.6) 36.7 (±1.8)	0.7	6.1
	rs4148553	Chr13 3' UTR	A G	44 56	AA AG GG	17 (22.3) 33 (43.4) 26 (34.3)	36.87 (±0.4) 36.89 (±0.7) 36.95 (±0.7)	0.9	42

PTGER2	rs45461592	Chr14 3' UTR	A T	69.7 3.3	AA TA	86 (93.5) 6 (6.5)	36.8 (±0.7) 37.1 (±0.3)	0.4	5
	rs708502	-	A G	21 79	AA AG GG	4 (5) 26 (32) 51 (63)	36.7 (±1.4) 36.8 (±0.7) 36.9 (±0.5)	0.9	28
	rs17197	Chr14 3' UTR	C T	14 86	CC TT TC	3 (3.5) 64 (75.3) 18 (21.2)	36.3 (±1.3) 36.8 (±0.5) 36.7 (±0.7)	0.22	28
PTGER3	rs3819783	Chr1 Intron	C T	24.4 75.6	CC CT TT	4 (4.4) 36 (40) 50 (55.6)	36.8 (±0.5) 36.6 (±0.7) 37.0 (±0.6)	0.055	0.244
PTGER4	rs16870224	Chr5 3' UTR	A G	19.6 80.4	AA AG GG	1 (1.5) 25 (36.2) 43 (62.3)	37.4 (0) 36.8 (±0.7) 36.7 (±0.7)	0.6	13.3
ADRB1	rs1801253	Chr10 Missense	C G	91.65 8.35	CC CG	45 (83.3) 9 (16.7)	37.02 36.7	0.06	29.4
ADRB2	rs1042714	Chr5 Missense	C G	52 48	CC GG GC	24 (34) 21 (30) 26 (36)	37.0 (±0.6) 36.9 (±0.7) 36.7 (±0.6)	0.2	24
ADRB3	rs4998	Chr8 3' UTR	C G	0.5 99.5	CG GG	1 (1) 89 (99)	36.7 (0) 36.9 (±0.6)	0.76	9.5
GABRG2	rs211014	Chr5 Intron	A C	29 71	AA CC CA	4 (6) 32 (48) 31 (46)	36.6 (±0.5) 36.8 (±0.5) 36.9 (±0.7)	0.6	38
	rs211035	Chr5 Missense	A G	28.5 71.5	AA AG GG	9 (12) 25 (33) 42 (55)	37.0 (±0.4) 36.8 (±0.5) 36.8 (±0.7)	0.5	20

HPGD	rs2253170	Chr4	A G	10 90	AG GG	12 (20) 49 (80)	37.0 (±0.3) 36.8 (±0.7)	0.2	5
	rs45439401	-	C T	23.5 76.5	CC TT TC	4 (5) 46 (58) 29 (37)	36.7 (±0.3) 36.8 (±0.6) 36.9 (±0.6)	0.5	?
CBR3	rs879894	Chr21 Intron	A C	36 64	AA CC CA	15 (16) 40 (44) 36 (40)	36.9 (±0.7) 37.0 (±0.5) 36.8 (±0.6)	0.4	0.39
ADRBK1	rs114509093	Chr4	C T	0.7 99.3	CT TT	1 (1.4) 68 (98.6)	37.3 (±0) 36.9 (±0.6)	0.47	0.084

4.2.2.2. The association between genotypes and increase in temperature with misoprostol

In the Liverpool population, the baseline temperature was less than normal in many women. Therefore, to overcome the possibility of false effect of this low baseline temperature on the final result of women's temperature after treatment with misoprostol, we examined the association of genetic polymorphisms with the change in temperature from the baseline. However, there was no association between the SNPs under investigation and change in the temperature in women who were treated with misoprostol in Liverpool.

4.3.3. Chills and/or shivering

We related the occurrence of chills and/or shivering to the genetic outcomes. There was no significant association between genetic polymorphisms under study and chills and/or shivering after treatment with misoprostol in the Ecuador and Liverpool populations.

5. Discussion

To our knowledge, this is the first study to examine the association between SNPs and the development of fever in women treated with misoprostol in two different populations predominantly were population from Liverpool and from Ecuador.

This study showed variations in the clinical data between these two populations. The incidence of fever ≥ 37.6°C in the Ecuador population was 90%, whereas in Liverpool population the incidence was about 9%. Moreover, 10% of the Ecuadorian population had fever higher than 40°C. The most common ethnicity in Ecuador population was Mestizo and in the Liverpool population it was White British. Variation in the incidence of fever was observed in different populations. Many studies have reported fever as a side effect of misoprostol, particularly in the treatment of PPH. A post hoc analysis study to explore the triggers of high fever in Ecuadorian women who were treated with misoprostol showed that 58 of 163 (35.6%) experienced fever ≥40°C (Durocher et al., 2010). Also, our study of the effect of misoprostol on postpartum contractions, which was conducted on a Libyan population, showed that 45% of the women had fever higher than 39°C (Elati et al., 2011). On the other hand, populations from Egypt, Turkey and Vietnam showed an incidence of high fever of less than 3% (Durocher et al., 2010). Hence, the genetic effect hypothesis has arisen.

Low incidence of fever in the Liverpool population may be explained by the difference in the route and dose of misoprostol and the gestational age during the treatment. High incidence of high fever was mainly reported in the postpartum period and with the sublingual route (Winikoff et al., 2010). However, the Liverpool population had vaginal misoprostol during the first trimester for TOP. A low incidence of fever (<3%) was also observed in a recent study in Ecuador for treatment of incomplete miscarriage using 600 mcg oral misoprostol (Montesinos et al., 2011). This may be explained by the increase in the production of prostaglandin during labour. In placenta, the PG synthase expression profile favours production of PGE_2 with high levels of PG transporters and catabolic PG dehydrogenase suggesting rapid PG turnover. Choriodecidua is primed for PGE_2 production whereas myometrium from non-pregnant women has lower levels of prostaglandin synthases than pregnant

myometrium (Phillips et al., 2011). Therefore, administration of misoprostol during the third stage of labour is adding to the endogenous prostaglandins and the overall level of prostaglandin is high compared to the levels in the first trimester. This increasing level of prostaglandins may contribute to the increasing incidence of temperature during the early postpartum period. On the other hand, low rates of fever were observed in some populations during the postpartum period and therefore, genetic polymorphisms may also have a role in the body response to misoprostol and the susceptibility of women to fever.

This study examined 33 SNPs in 14 genes related to prostaglandin's pathway. Two genes were involved in prostaglandin metabolism receptors, three prostaglandin transporters and 9 prostaglandin target genes. Two polymorphisms in the prostaglandin transporter SLCO1B1 (rs4149087 and rs4149085) were associated with misoprostol-induced fever in the Ecuador population. Also, there was a polymorphism (rs114869610) in SLCO2A1 transporter gene associated with misoprostol-induced fever. Genetic polymorphisms in prostaglandin transporters were found to be associated with different diseases' susceptibility and drug responses. For instance, the polymorphism rs4149056 in SLCO1B1 is associated with decreased intake of anti-cancer drug SN-38 from the systemic circulation, leading to an increase in its plasma concentration and enhancing the risk of neutropenia (Takane 2011). This SNP is also associated with statin-induced adverse drug reactions. It reduces the uptake of simvastatin acid into the hepatocytes (where it inhibits cholesterol synthesis) and results in an increase in its plasma concentration and enhancing the risk of myopathy (Niemi, Pasanen & Neuvonen 2011). However, the SNPs we found to be associated with misoprostol-induced fever in the Ecuador population have not been shown before to be associated with any drugs' side effects.

The allelic frequency of SLCO1B1 (rs4149087) in healthy Ecuadorian population was 0.4 (Bryc et al., 2010)[1] which is very close to the allelic frequency in our population of Ecuadorian women who were treated with misoprostol for PPH (0.35).While we had a small sample size, it can be representative of the Ecuadorian population. Also, the allelic frequency of SLCO1B1 (rs4149087) in a healthy Colombian population is

[1] Genotype data from 100 Hispanic/Latinos deposited in the gene expression omnibus (GEO) series record GSE21248.

almost similar to the allelic frequency in our population, which was (0.36) (Bryc et al., 2010)[1]. Therefore, the result of this study may suggest that misoprostol-induced fever may also occur in Colombian women if they were treated with misoprostol.

The finding of this study may contribute to the previous suggestion of the hypothesis of misoprostol-induced fever. In normal situations (in the absence of PGE_2), EP3-expressing neurons produce a strong GABAergic tonic inhibition on fever-mediated raphe pallidus neurons (rRPa) and DMH (dorsomedial hypothalamus) neurons. Genetic polymorphisms in prostaglandin transporters' genes (SLCO1B1 & SLCO2A1) may lead to an increase in the expression of prostaglandin transporters receptors (influx receptors) in the blood-brain barrier (BBB). This increases the uptake of prostaglandin (misoprostol) by the BBB to the hypothalamus. PGE_2 suppresses the GABAergic activity of EP3-expressing neurons, resulting in disinhibition and activation of the rRPa and DMH neurons in the hypothalamus, leading to thermogenesis (Nakamura et al., 2005) (Figure 15).

This study has highlighted the genetic polymorphisms association between clinical phenotype (fever) and misoprostol treatment. It is the first in this subject and may show a good example of research into population-based treatment strategies. However, further exploration is required using a large sample size and different populations. In populations with misoprostol-induced fever, particularly more than 40°C, the use of misoprostol for PPH may be withheld because of this side effect. Genetic testing for individuals' susceptibility to misoprostol-induced fever is not cost effective with the wide use of misoprostol for PPH in some populations. However, this may be done based on wide population genetic testing. Samples from populations with a high susceptibility to fever can be examined genetically for the presence of prostaglandin transporters' polymorphisms and for populations which show these polymorphisms, treatment could be modified in terms of dosages and routes of administration while preserving the drug's efficacy.

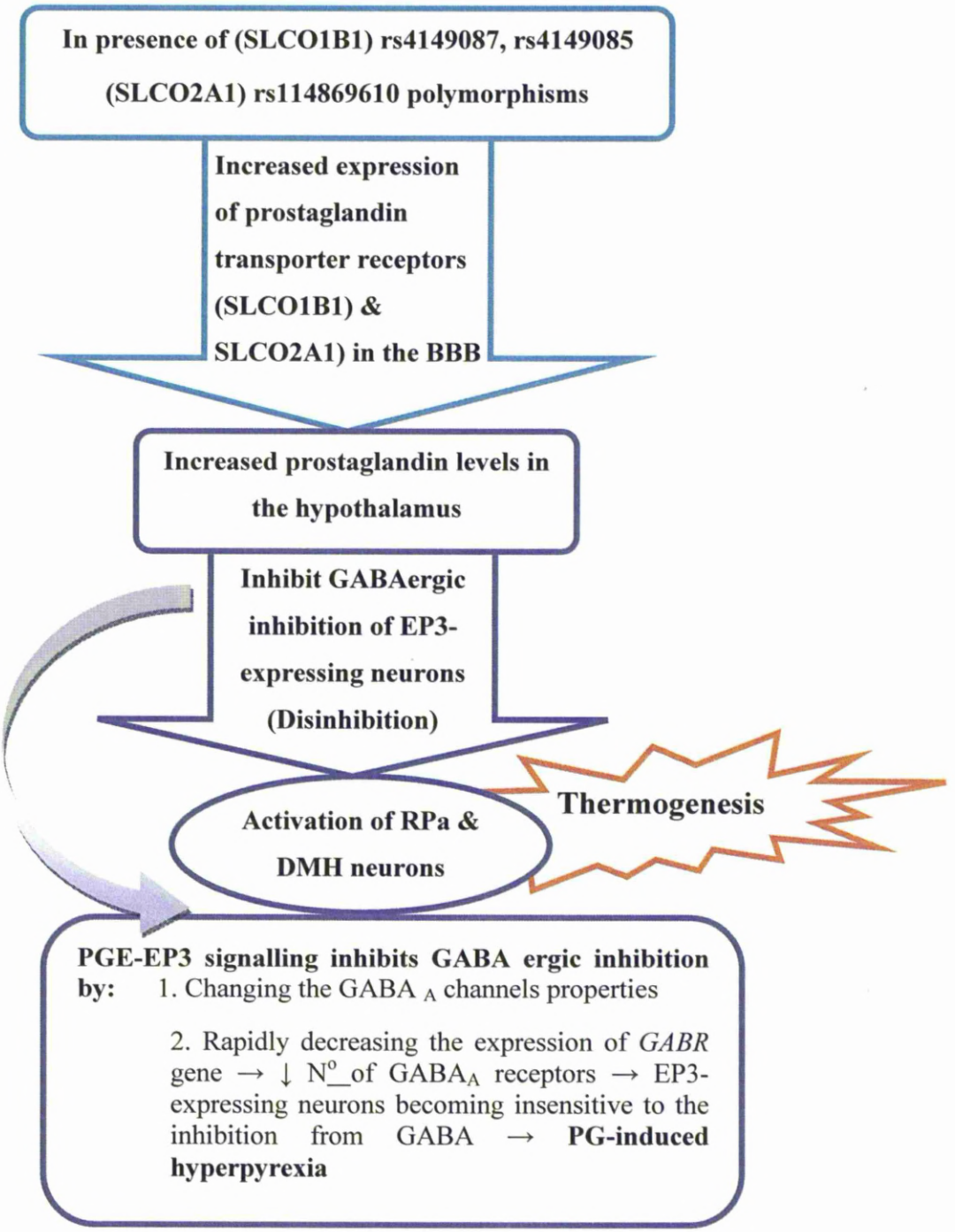

Figure 15. The suggested mechanism of misoprostol-induced fever

There were several limitations to the study that the genetic association with misoprostol-induced fever could be addressed much better with a larger sample size from Ecuador and Liverpool populations. First, the Ecuadorian group was part of another clinical trial for treatment of PPH where we obtained all the blood samples from the women who had been treated with misoprostol and more samples cannot be obtained. Second, in Liverpool, there was a big hindrance with recruitment of women to the study as they were treated with misoprostol for TOP. The sensitivity of this issue made recruitment difficult and complicated. The Bedford Clinic in Liverpool has good admission rate per week. However, unexpectedly, the recruitment was very difficult, as our study does not involve any aspect of their treatment. In addition, many women were unhappy to give a blood sample. We tried to overcome this problem by taking the study sample while they were giving blood for the investigations required by the hospital on the consultation visit and tracing them on the day of admission to fill in the case report form and to measure the temperature. Tracing the women was not easy as many of them have been missed due to many reasons. Some women cancelled before the day of admission and some of them were rescheduled for admission. Registration of the women in the trial took place after signing the consent, which took place either on the consultation visit or on the day of admission for misoprostol treatment. We recruited 107 women (who signed the consent) and we had complete data and blood samples from 93 of them. Also, changes in the Bedford Clinic schedule between medical and surgical TOP compromised the recruitment to some extent. The genotypic frequencies of the identified polymorphisms were low. Although we have included some SNPs with rare alleic frequencies, they were of known functional significance and expected to have a large effect.

Our aim was to do a case-control study for the Ecuador and Liverpool populations. But, unexpectedly, we found that most of the Ecuadorian women had fever with misoprostol (46/50) and there were few controls, and the contrary happened in Liverpool population where we had few cases (8/93) and a large number of controls (85/93). Therefore, we analysed our data in a single group for each population using the analysis of continuous variables (ANOVA). It would be important to compare the genetic association of Liverpool and Ecuador populations but this was unfeasible for the following reasons. The two populations have different diseases' phenotype and

also they have different doses and routes of treatment. The Ecuadorian women had 600 mcg sublingual misoprostol as stated in the protocol of the clinical trial, while Liverpool women received 800 mcg vaginal misoprostol. Also, those two populations included different ethnic groups. The Ecuadorian population was predominantly Mestizo, whereas the Liverpool population was mainly white (Caucasian). The inconsistency of the doses and routes of administration, the disease phenotype and population structure made the comparison impractical. Therefore, we considered each population separately when dealing with the data analysis.

The Ecuador population was selected deliberately to be examined because of the high incidence of fever and the results may be extrapolated to other populations. However, in order to detect a true genetic association we need to perform this study on a larger population with clear characterisation of the phenotype and the ethnicity. In this study, we had 50 samples from women from Ecuador. The power calculation showed that we had a strong chance to find the difference in our sample of 2-3 degrees C for all SNPs with the allelic frequency above 20%. Based on the following assumptions: Alpha-level=0.002 (0.05/33), mean in wild-type group: 37 degrees, standard deviation: 0.85

		Minor allele frequency		
		0.05	0.20	0.40
Difference (degrees) between WT group and mutant-type group	1	11%	52%	94%
	2	74%	99%	99%
	3	99%	99%	99%

The calculations were performed in nquery.

Therefore, we had 99% power to detect the difference of 2 degrees C for SNPs with minor allele frequency of 20%. For SNPs with lower frequencies (approx 5%) we had 99% power to detect the difference of 3 °C. We took into consideration the number of SNPs tested (N=33) and corrected for multiple comparisons using Bonferroni correction.

For future case-control study, depending on our findings in this study, the incidence of fever ≥ 40 °C was 10%, the allelic frequency of the positive genetic association SNP (rs 4149087) was 35%, the sample size for a case control study with 80 % power will be 60 case and 60 controls (figure 16). The calculation was done using GPA calculator from

http://www.webcitation.org/query.php?url=http://dceg.cancer.gov/bb/tools/pga&refdoi=10.1186/1471-2156-9-36

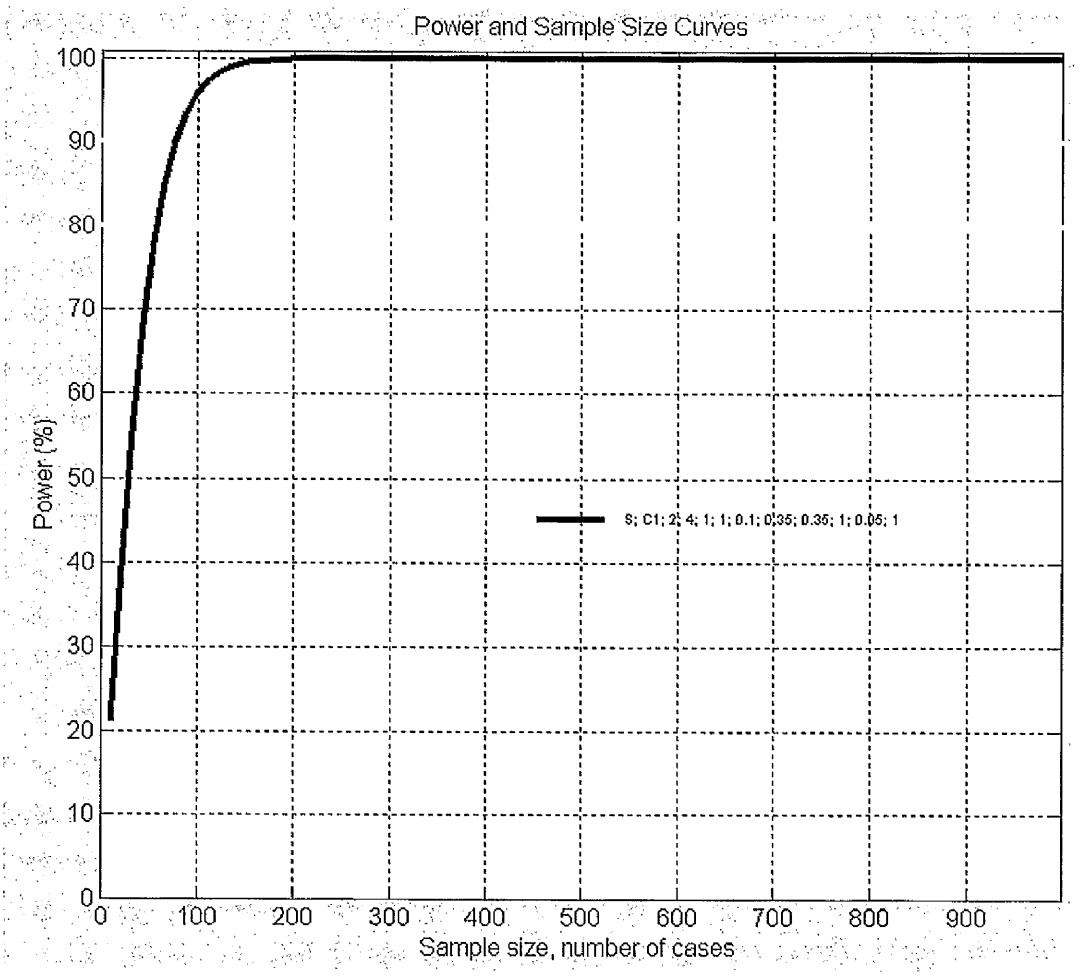

Figure 16. The sample size calculation curve for a genetic association case control study

6. Conclusion

The incidence of fever has shown clear variations between the Liverpool and Ecuador populations in women treated with misoprostol. To our knowledge, this hypothesis generating study is the first study to investigate the genetic association between polymorphisms in genes encoding prostaglandin transporters and misoprostol-induced fever in women treated for PPH.

Chapter 5

General discussion and future research

1. General discussion

The overall work in this thesis aimed to optimise the use of misoprostol for the prevention of PPH. The literature review showed the lack of clear evidence for what is the most effective and safest dose and route of misoprostol for prevention of PPH. Skilled and non-skilled birth attendance tend to use high doses of misoprostol in the hope of achieving the strongest effect and this may result in many unwanted adverse drug reactions. Therefore, we hypothesised that lower doses of misoprostol may achieve a similar effect on the uterine muscle contractility while maintaining a good side effects profile.

It is important to reach the best balance between the efficacy and the safety of any recommended treatment. The systematic review and the meta-analysis in Chapter 2 showed that the use of misoprostol was associated with a high incidence of fever particularly with high doses of sublingual misoprostol. However, there was wide heterogeneity between the trials in this review. This could be explained by the shortage of data about the side effects in the clinical trials but might also be related to the mixed populations and ethnicity in the studies. Ethnic difference are supported by, data from a multi-centre randomised control trial that used 800 mcg sublingual misoprostol for the treatment of PPH. It found that the incidence of high fever (>40°C) in the Ecuador population was significantly higher than the incidence with the other populations (Winikoff et al., 2010). This has raised the possibility of a genetic association with misoprostol-induced fever. Therefore, a trial was conducted to findout the best effective dose of sublingual misoprostol to reduce the incidence of fever.

In Chapter 3, the validity of the IUPCs, which were produced to measure the IUP during the first stage of labour, was investigated. Previous studies had been conducted using transducer-tipped catheters (Intran plus), but this was unlikely to be accurate outside of a closed air or fluid system. The validity of IntranPlus catheter was therefore compared with a new balloon catheter (Koala). The study however, showed neither of the two types of the IUP catheters (Koala E5000 and Intrans plus) were valid outside of a closed pressure system. The Koala was however the most reliable.

We therefore used the Koala for the clinical trial. We rationalised that we are not interested so much in the exact amount of pressure produced, but wished to compare the effect of variable doses of sublingual misoprostol on the uterine muscles. The study of the effect of misoprostol on postpartum uterine contraction of three sublingual doses of misoprostol showed that 200 mcg, 400 mcg and 600 mcg doses of sublingual misoprostol had significant effect on the uterine muscle activity but that the severity of the side effects was dose dependent. The result of this study provided good evidence that lower doses of misoprostol might be effective clinically but with fewer side effects. Our findings were supported by a recent randomised clinical trial, investigating the effect of 400 mcg sublingual misoprostol for prevention of PPH, which cited our paper (Chaudhuri, Biswas & Mandal 2012). We expect more research using lower doses of misoprostol and the findings of our study will be added to the body of evidence supporting a new recommendation of using 400 mcg sublingual misoprostol for the prevention of PPH instead of the current recommendation, which is 600 mcg of oral or sublingual misoprostol (Weeks & Faundes 2007).

In Chapter 4, the genetic association with misoprostol induced fever was explored. Three single nucleotide polymorphisms (SNPs) in two genes encoding for the prostaglandin transporters (SLCO1B1 and SLCO2A1) were discovered to be associated with misoprostol-induced fever in the Ecuadorian population. Although the sample size was small, this finding highlighted the possibility of the genetic variation as a cause of the high fever in certain population. Physiologically, the presence of these SNPs in the prostaglandin transporters, which expressed in the blood brain barrier may cause more efflux of misoprostol into the hypothalamus and thus increase the thermoregulatory set-point to a higher temperature. Clinically, misoprostol may be omitted from the management of PPH in populations where these SNPs are common, or the protocol should be tailored towards lower doses. The genetic role hypothesis might not be the only mechanism that explains the causes of fever.

2. Future research

Inevitably, whenever research questions are answered, further questions arise. This increases the researchers' curiosity and may lead them to continue their investigations and improve the methodology, or to replicate their previous findings. The work in this thesis could open new avenues in several aspects of research around optimising the use of misoprostol and understanding its local and systemic effects on the human's body.

Although the use of intrauterine pressure measurements was well established many years ago, the uterine activity during the third stage of labour requires more research. This may involve research into producing a new catheter with a larger sensitive surface area or measuring the effect abdominally using sensitive sensors to detect the change in electric myoactivity. The in vitro experiment in Chapter 2 could be replicated in vivo in the optimum condition of a post partum uterus and test the reliability of the IUP catheters.

The close observation of postpartum women who were treated with misoprostol in Libya showed how these side effects are very irritating and unacceptable, even though medically they were not very serious. All women after being exhausted in labour and delivery are seeking a relaxed and peaceful environment to rest. But severe shivering and fever at this time will adversely affect their early postpartum hours. Therefore, more research is essential to find a suitable misoprostol dose and route with minimal side effects to be used particularly in populations at risk of developing the side effects. A multi-centre randomised control trial may provide a good opportunity to document the rates of shivering and fever, and to collect blood samples to replicate the genetic study in Chapter 4. The data could also increase the evidence towards new recommendation of lower doses of misoprostol. Moreover, the use of the misoprostol tablet which was produced for swallowing is not practical for the sublingual route. After sublingual administration of misoprostol, the unabsorbed vehicle (cellulose) of the drug may stay in the mouth for long time and delay the absorption of the active ingredients. Producing a new form of misoprostol such as oral lyophilisates (oral melt) may provide a good alternative to the current tablets both in practicality and speed of absorption.

The pharmacogenetic study of misoprostol induced fever needs replication using samples from a large multi centre randomised control trial comparing high and low doses of misoprostol. This could result in many samples from diverse populations and adequate power to confirm or refute the genetic findings. Also, it needs to take in consideration the characterisation of the selected populations in order to have a good powerful association if it has to occur.

Is misoprostol a substrate for the prostaglandin transporters in the brain? How does it pass the blood brain barrier? These are some of the questions which need an answer. Examination of the movement of misoprostol across a blood brain barrier cell line using radio-labelled misoprostol acid may provide us with information that can support the genetic hypothesis. It would be even more exciting if we could examine and test blood brain barrier cells from people with known genetic variations in genes encoding the prostaglandin transporters (SLCO1B1 and SLCO2A1). This would be very ambitious and would require further confirming data regarding the genetic variations before it was undertaken.

Prevention of PPH is an important subject and research is going on all over the world to improve the current management and medications. Misoprostol is clearly a promising drug which can help reducing PPH, particularly in low resource settings. The work in this thesis has contributed to the research into misoprostol for the prevention of PPH in terms of finding the suitable dose of misoprostol which provides the required efficacy while maintaining a good side effect profile. As a result we recommend the use of 400 mcg sublingual misoprostol for prevention of PPH, a dose that balances proven efficacy with an improved side effect profile. This dose may need to be reduced further in those populations which have high prevalence of the genetic polymorphisms in SLCO1B1 and SLCO2A1 genes.

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Appendixes

Appendix A. 1. Data Extraction Form

Systematic review of misoprostol induced fever
Data Extraction Template (RCT)
Trial details

Trial methods

Trial ID		Extractor		Year of publication	
Title					
Authors					
Duration of study		Single or multicentre study			
Method of allocation generation	Describe Adequate / unclear / inadequate				
Method of allocation concealment	Describe Adequate / unclear / inadequate				
Loss of participants	Describe <5% 5-10% 10-20% >20%				
Blinding	Participants	yes / no / unclear			
	Clinician	yes / no / unclear			
	Outcome assessor	yes / no / unclear			
Intention to treat analysis	Describe Used / unclear / not used				

Participants

Study population (ethnicity) & location (hospital or community)	
Inclusion criteria	

Exclusion criteria	

Intervention

Indication of treatment

	Study medication	Dose	Route	Pregnancy stage
Experiment group1				
Experiment group2				
Experiment group3				
Control group				

Additional information about (fever)

Definition of fever		Described	Not described
Definition of fever	(Categories)	Mild Moderate Severe	
Method of monitoring fever		Reported	Not reported
Method of monitoring the temperature is		oral	axillary
How was fever data collected?		Prospective/ routine monitoring Patient check-list/ questionnaire Not describe	

Outcomes

Outcome measures	Total women =							
	Intervention group 1 =		Intervention group 2=		Intervention group 3=		Control group =	
	Events	incidence	Events	incidence	Events	incidence	Events	incidence
Temp>								
Temp>								
Temp>								
Unspecified fever								
Chills/ Shivering								
Chills & fever unspecified								

Appendix A. 2. Characteristics of the included studies

Study	Methods	Participants	Interventions	Outcomes	Notes	Allocation concealment	Quality of reporting side effects
Amant 1999	Randomized double blind controlled trial using computer generated list. The study boxes and capsules were indistinguishable.	Women who had SVD. The exclusion criteria were caesarean delivery, hypertension, gestational age <32 weeks, intrauterine death, uterine malformations, allergy to prostaglandins or alkaloids, inflammatory bowel disease and sepsis.	600 mcg oral misoprostol or 200 mcg methyl-ergometrine.	The main outcomes were the rates of PPH, need for therapeutic oxytocic drugs and side effects.	Intention to treat analysis with less than 10% loss of participants to follow up. The study was conducted in Belgium over 5 months.	B	Good
Baskett 2007	Randomised controlled trial using computer generated randomisation cards which kept in sealed, opaque, sequentially numbered envelopes.	Women with singleton fetus, cephalic presentation in spontaneous or induced labour. The exclusion criteria were placenta praevia, abruptio placenta, coagulation disorders, unstable asthma, and caesarean delivery.	400 mcg oral misoprostol or 5 IU oxytocin IV.	The primary outcome was a haematocrit drop of 10% or greater over the first 24 hours postpartum. The secondary outcomes were haemoglobin drop of 30 mg/L or greater, the administration of additional oxytocic drugs, an estimated blood loss>	Intention to treat analysis with no loss of any of the participants. The study was conducted in Canada over 3 and half years.	A	Good

				1000 ml, manual removal of placenta, blood transfusion , shivering and fever≥ 38 °C.			
Caliskan 2002	Randomised controlled trial using computer based random allocation without any blocking or stratification. The list of medication was printed out and put in sealed, consecutively numbered opaque envelopes.	Women in labour. Exclusion criteria was caesarean delivery gestational age >32 weeks and known hypersensitivity to prostaglandins.	500 mcg rectal misoprostol and 10 Iu oxytocin infusion or 500 mcg rectal misoprostol and placebo or 10 Iu oxytocin infusion and placebo or 200 mcg methyl ergometrine Im and oxytocin 10 IU infusion.	The primary outcomes were the incidence of PPH and the decrease in the Hb concentration . The secondary outcomes were the incidence of severe postpartum haemorrhage, mean blood loss, need for additional uterotonics incidence of post partum blood transfusion, length of the third stage of labour and specific side effects.	Intention to treat analysis with less than 5% loss of participants to follow up. The study was conducted in Turkey over 10 months.	A	Good
Caliskan 2003	Randomised controlled trial using computer based random allocation without any blocking or stratification. The list of medication was printed out and put in sealed, consecutively numbered	Women in labour. Exclusion criteria was caesarean delivery gestational age >32 weeks and known hypersensitivity to prostaglandins.	600 oral misoprostol and 10 Iu oxytocin infusion or 600 mcg oral misoprostol and placebo or 10 Iu oxytocin infusion and placebo or 200 mcg	The primary outcomes were the incidence of PPH and the decrease in the Hb concentration . The secondary outcomes were the incidence of severe	Intention to treat analysis with less than 15% loss of participants to follow up. The study was conducted in Turkey over 10	B	Good

	opaque envelopes.		methyl ergometrine Im and oxytocin 10 IU infusion.	postpartum haemorrhage, mean blood loss, need for additional uterotonics incidence of post partum blood transfusion, length of the third stage of labour and specific side effects.	months.		
Chhabra 2008	Randomised controlled trials using random number tables in group of 100 each. The concealment method was not reported in the paper.	Women with term gestation and spontaneous onset of labour. Exclusion criteria were grand multiparity (parity >5), multiple gestation, pregnancy induced hypertension, APH, labour induction or augmentation, past or planned caesarean section, Hb<8gm/Dl or obstetrics problems and known hypersensitivity to prostaglandins.	100 mcg oral misoprostol or 200 mcg oral misoprostol or 200 mcg methyl-ergometrine IV.	Amount of blood loss, need for additional oxytocic, third stage complication and any side effects.	Intention to treat analysis with no loss of any of the participants . The study was conducted in India.	B	Good
Cook 1999	Multicentre randomised trial using random number list in blocks of 20 with a separate randomisation for each centre and sequentially numbered sealed security opaque envelops.	Women who expecting a vaginal delivery. Exclusion criteria: women with known blood coagulation disorders, a history of asthma, heart disease, severe renal disease or epilepsy, women	Misoprostol 400 mcg orally, IM10 IU oxytocin or syntometrin e.	Primary outcomes, uterine blood loss, need for uterine message, the use of additional uterotonics, the need for blood transfusion. Secondary	Intention to treat analysis with less than 10% loss of participants to follow up. The study was conducted in	A	Poor Definit ion of fever and metho d of data collecti on were not

		undergoing an elective caesarean section and women with hypertension.		outcomes, changes in temperature, pulse and blood pressure, vomiting, diarrhoea, shivering, Hb level 24 hrs postpartum.	Australia.		reported
El-Refaey 2000	Randomised controlled trial using computer-generated block randomisation with varying block size. The concealment was by opaque, sequentially numbered sealed envelopes	Women who had normal vaginal delivery. The exclusion criteria were caesarean delivery, history of bronchial asthma and water birth.	500 mcg oral misoprostol or 10 IU oxytocin or syntometrine or 500 mg ergometrine.	Incidence of PPH and the incidence of the severity of the side effect	Intention to treat analysis with less than 5% loss of participants. The study was conducted in London over 24 months.	A	No clear definition. Routine monitoring of fever
Enakpene 2007	Randomised controlled trial using simple random selection.	Women with singleton pregnancy, low risk pregnancy, vertex delivery. The exclusion criteria were the presence of contraindications to the use of either misoprostol or methylergometrine such as pre-eclampsia and other hypertensive disease in pregnancy, pre-existing cardiac disease, severe anaemia, history of asthma, renal or hepatic disorders, allergy to prostaglandin and presence of	400 mcg oral misoprostol or 500 mcg intramuscular methyl-ergometrine.	The primary outcomes were estimated blood loss during delivery and within the first 24 hours postpartum, the duration of the third stage of labour, and additional use of oxytocics. The secondary outcomes were maternal vital signs, adverse effects such as nausea, vomiting,	Intention to treat analysis with no loss of any of the participants. The study was conducted in Nigeria over 1 year.	A	Good

		condition requiring prophylactic oxytocin infusion.		headache, chest pain, abdominal cramps, fever, shivering.			
Garg 2005	Randomised controlled trial using 1:1 ratio by random number sequence.	Women with singleton pregnancy and have SVD.	600 mcg oral misoprostol or 200 mcg methylergometrine IV.	Amount of blood loss, haemoglobin concentration, duration of the third stage of labour, manual removal of placenta, need of additional oxytocic. Side effects such as nausea, vomiting, diarrhoea, headache, fever ≥ 38 °C, shivering and vertigo.	Intention to treat analysis with no loss of any of the participants. The study was conducted in India over 1 year.	B	Poor The method of data collection was not reported
Gülmezoglu 2001	Multicentre randomised controlled trial using computer regenerated numbers and identical treatment packs, sealed and numbered sequentially and could only be taken from the dispenser consecutively.	Women in labour. The exclusion criteria were women with asthma or other severe chronic allergic conditions, planned caesarean section, body temperature > 38 °C.	600 mcg oral misoprostol or 10 IU oxytocin IV or IM.	The primary outcomes were measured postpartum blood loss of 1000 ml or more and the use of additional uterotonics. The secondary outcomes were measured blood loss more than 500 ml, blood transfusion, manual	Intention to treat analysis with no loss of any of the participants. The study was conducted over 1 year and 9 months.	A	Good

				removal of placenta, complications such as bimanual compression, hysterectomy, suturing of cervical tears and maternal admission to intensive-care unite.			
Gupta 2006	Double blind randomised pilot study. Randomisation by using computer generated random tables. The sealed envelope with a code number was opened when vaginal delivery is imminent.	Women in labour	600 mcg rectal misoprostol or 10 IU oxytocin	The primary outcomes were the incidence of PPH and the decrease in the Hb concentration. The secondary outcomes were the incidence of severe postpartum haemorrhage, mean blood loss, need for additional uterotonics, need for bimanual removal of placenta, length of the third stage of labour and specific side effects.	Intention to treat analysis with less than 5% loss of participants to follow up.	A	Good
Harriott 2009	Randomised controlled trial using computer generated block randomisation. The treatment was based on the assigned number being either IV	Women who is expecting to a vaginal delivery. Exclusion criteria: previous PPH, hypertensive disorders, previous caesarean section,	400 mcg rectal misoprostol, oxytocin 10 IU and 0.5 mg ergometrine.	Outcome, blood loss, additional oxytocic therapy. Side effects.	Intension to treat analysis with less than 5% loss of participants.	A	Good

	syntometrine or rectal misoprostol.	intrauterine fetal death, ant partum haemorrhage, anaemia (Hb< 8g/l)					
Hofmeyr 2001	Randomised, placebo-controlled trial using computer generated random sequence, in balanced block of 18 and a serious of numbered opaque test tubes.	Women in labour.	600 mcg misoprostol orally or placebo.	Primary outcomes, shivering, pyrexia ≥ 37.8 C and hypertension. Secondary outcomes, nausea, vomiting, diarrhoea, abdominal pain, blood loss ≥ 1000 ml, additional use of oxytocic, manual removal of placenta and blood transfusion.	Intention to treat analysis with no loss of any of the participants.	A	Poor Method of data collection was not reported
Høj 2005	Randomised double blind controlled trial using a list of random numbers and consecutively numbered opaque envelopes.	All women in labour.	600 mcg sublingual misoprostol or placebo.	The primary outcome was postpartum haemorrhage of ≥ 500 ml, change in Hb concentration, the incidence of 10% decrease in haemoglobin concentration. Side effects such as nausea, diarrhoea, vomiting, shivering and rectal temperature.	Intention to treat analysis with no loss of any of the participants. The study was conducted in Guinea-Bissau over one and half year.	A	Good

Kundodyiwa 2001	Randomised controlled trial using computer generated numbers using random sequence and sealed opaque envelopes.	Women with singleton pregnancy at term (>37 weeks of gestation). The exclusion criteria were a history of PPH, condition causing disseminated intravascular coagulation, antepartum haemorrhage, coagulation disorders, operative delivery, multiple pregnancies, history of asthma, and known allergy to prostaglandin and oxytocin.	400 mcg oral misoprostol or 10 IU oxytocin IM.	The main outcome was the incidence of PPH. The secondary outcomes were need for additional uterotonics, need for blood transfusion, manual removal of placenta, duration of the third stage of labour, need for subsequent evacuation of the uterus.	Intention to treat analysis with less than 1% loss of participants to follow up. The study conducted in Zimbabwe over 5 months.	A	Good
Khan 2003	Randomised controlled trial using computer-generated random numbers with blocked randomisation. consecutively numbered sealed opaque envelopes each contains a fold slip of paper with the treatment written on it.	Women with singleton pregnancy at term (>37 weeks of gestation). Exclusion criteria were women with asthma, or other chronic conditions, cardiac disease, renal failure, renal or hepatic disorders.	600 mcg oral misoprostol, 400 mcg rectal misoprostol and 600 mcg rectal misoprostol	The concentration of misoprostol free acid The primary outcome for the adverse-effect profile study was shivering as experienced by the patient. Adverse effect as observed by the birth attendant. Oral measurement of temperature.	Intention to treat analysis with less than 1% loss of participants to follow up.	A	Good
Lokugamage 2001	Randomisation using computer-generated random number	Women undergoing elective and emergency	500 mcg oral misoprostol or 10 IU IV	Estimated blood loss at caesarean section, drop	Intension to treat analysis with less	A	Good

	and the concealment was by sealed opaque envelopes.	caesarean section. Exclusion criteria: two or more previous sections, history of rupture uterus.	syntocinon	in serum haemoglobin and the need for additional uterotonics agents.	than 5% loss of participants .		
Lumbiganon 1999	Multicentre randomised controlled trial. Method of randomisation was not described. Concealment by using treatment packs sealed numbered and could only taken from the dispenser consecutively.	Exclusion criteria were women with asthma, or other severe chronic allergic condition, contraindication to misoprostol planned caesarean section.	600 mcg oral misoprostol or, 400 mcg oral misoprostol or 10 IU oxytocin intramuscular.	The incidence of shivering. Any side effects.	Intention to treat analysis with less than 2% loss of participants to follow up.	B	Good
Miller 2009	Double blind randomised controlled trial using computer generated randomisation list with random block size. The concealment by sealed opaque envelopes contains either active drug or placebo.	18 years old, singleton pregnancy, > 28 weeks pregnancy. The exclusion criteria: previous or planned caesarean section, pre-eclampsia, severe anaemia, history of bleeding disorders, mental disability, body temperature >38°C, serious medical illness, active haemorrhage st the time of screening, women with asthma or glaucoma, women with severe labour pain (to be eligible for informed consent).	600 mcg oral misoprostol or Zhi Byed 11 a Tibetan traditional medicine.	The incidence of PPH, administration of uterotonics or maternal death.	Intention to treat analysis with less than 5% patient loss.	A	Poor no clear definition of fever, method of data collection about fever was not described.

Nasr 2009	Double blind randomised controlled trial using computer-generated random allocation system and sealed opaque consecutively numbered envelopes.	All women who have spontaneous vaginal delivery of a live, singleton neonate, and absences of any contraindications for misoprostol or oxytocin use. The exclusion criteria were women who delivered by caesarean section, history of APH or bleeding tendency, hypertension with pregnancy and need for anticoagulants.	800 mcg of rectal misoprostol and placebo ampoule or rectal placebo and 5IU oxytocin infusion.	The primary outcome is the number of patents estimated to have PPH. The secondary outcomes were a haematocrit drop of 10% or more, haemoglobin concentration , change in systolic and diastolic blood pressure, duration of the third stage of labour, need for manual removal of placenta, nausea, shivering and fever $\geq$ 38°C.	Intention to treat analysis with no loss of any of the participants . The study was conducted in Egypt.	A	Good
Ng 2001	Multicentre randomised controlled trial. Randomisation was based on a table of computer generated blocks of random numbers.	Women who have singleton pregnancy and vaginal delivery. Exclusion criteria were the presence of contraindications to misoprostol or syntometrine, such as PET, cardiac disease and asthma of presence of conditions requiring prophylactic oxytocin infusion such as grnd multyparity (parity >4), or presence of	600 mcg oral misoprostol or 1 ampoule syntometrin e.	The primary outcome was the amount of blood loss. The secondary outcomes were change in maternal haemoglobin, maternal blood pressure, pulse and temperature, occurrence of side effects such as nausea, vomiting, headache, chest pain,	Intention to treat analysis with no loss of any of the participants . The study was conducted over 1 year.	A	Good

		uterine fibroids and women receiving oxytocin infusion during the first stage of labour.		fever, shivering.			
Ng 2007	Double blind randomised controlled trial using a table of computer generated random numbers and consecutively numbered sealed opaque envelopes.	All women having singleton pregnancy > 34 weeks of gestation, low risk for PPH and vaginal delivery. The exclusion criteria were presence of contraindications for the use of misoprostol or syntometrine such as PET, cardiac disease and asthma. The presence of conditions requiring prophylactic oxytocin infusion such as multiparity (≥ 4) or presence of fibroids and women received oxytocin in the first stage of labour.	400 mcg oral misoprostol or 1 ampoule syntometrine.	The primary outcome was the change in haemoglobin level. The secondary outcomes were the maternal blood pressure, pulse, temperature. Side effects including nausea, vomiting, headache and shivering.	Intention to treat analysis with no loss of any of the participants. The study was conducted in Hong Kong over 10 months.	A	Good
Oboro 2003	Randomised controlled trial using random number generated tables and opaque sealed packets containing cards indicating group allocation.	Women with SVD. The exclusion criteria were women who had caesarean delivery, HB < 8g/dl, risk factors for PPH (previous history of PPH, multiple gestation, polyhydraminos, grandmultiparity, induced or augmented	600 mcg oral misoprostol or 10 IU oxytocin intramuscular.	The primary outcome was the incidence of PPH. The secondary outcomes were side effects nausea, vomiting, shivering and elevated temperature.	Intention to treat analysis with no loss of any of the participants. The study was conducted over 1 year.	A	Good

		labour, uterine leiomyoma and precipitated labour.					
Parsons 2006	Randomized placebo controlled trial using computer generated randomization and sequentially numbered, opaque sealed envelops	All women presenting in labour including women at high risk of PPH. Exclusion criteria were any contraindication to prostaglandin such as known hypertension, asthma and epilepsy.	800 mcg oral misoprostol at the delivery of the anterior shoulder. The control group received 10 IU IM oxytocin.	Postpartum haemoglobin concentration , additional blood loss, occurrence of shivering, temperature >37.5 C	Intention to treat analysis with no loss of any of the participants . Study was done in Ghana, west Africa, over 6 months.	A	Good
Parsons 2007	Multicentre randomised controlled trial. The randomisation method was unclear; using opaque sealed envelopes containing a standard data sheet with a random assignment to either the control group and the treatment group.	All women presenting in labour. The only exclusion criteria were any known contraindication to prostaglandin randomisation.	800 mcg misoprostol rectally or 10 IU oxytocin IM.	Subject estimation of blood loss, change in haemoglobin concentration , occurrence of shivering, temperature monitoring,	Intention to treat analysis with less than 5% loss of participants to follow up. The study was conducted in Ghana over 9 months.	B	Good
Patted 2009	Multicentre randomised placebo controlled trial using computer generated randomisation list with a random block size and non-distinguishable envelops congaing	Pregnant women anticipated an uncomplicated vaginal delivery at $\geq$ 28 weeks. Exclusion criteria: previous or planned caesarean section, Hb<8g/L, anti-partum haemorrhage, hypertension,	600 mcg misoprostol orally or placebo.	Side effects, nausea, vomiting, shivering, fever, diarrhoea.	Intention to treat analysis with less than 1% loss of participants to follow up. The study was conducted in India over 3	A	Definit ion of fever was not reporte d

	misoprostol or placebo.	multiple pregnancy, history of APH, PPH, retained placenta, uterine inversion, diabetes, heart disease, seizures, breech, history of asthma.			years.		
Singh 2009	Double blind randomised trial using computer generated random number chart and sealed and coded drug packets.	Women with healthy singleton pregnancy in spontaneous or induced labor at term. Exclusion criteria: known hypersensitivity/contraindication to prostaglandins, intrauterine fetal demise, antepartum haemorrhage, multiple, pregnancy, malpresentation, cardiac disease, Rhesus-negative mother, hypertensive disorders and severe anaemia (Hb<7 g/dl)	400 mcg sublingual misoprostol, 600 mcg sublingual misoprostol, 5 IU oxytocin IV or 200mcg methyl ergometrine IV.	Amount of blood loss. Maternal pulse, blood pressure and temperature.	Intention to treat analysis with no loss of any of the participants. Study was done in India.	A	Good
Vaid 2009	A randomised controlled trial using computer generated random number. Method of allocation concealment was unclear.	Pregnant women ≥ 32 weeks with spontaneous or induced labour. The exclusion criteria were grand multi para (≥ 5), multiple gestation, < 32 weeks of gestation, HELLP syndrome, hydramnios, known blood coagulation disorders, history of asthma or drug	400 mcg sublingual misoprostol, 200 mcg IM methyl ergometrine or 125 mcg IM 15 methyl-PGF2α.	The primary outcomes were, the amount of blood loss, in the third stage of labour, PPH (blood loss >500 ml). The secondary outcomes were change in haemoglobin, need for	Intention to treat analysis with no loss of any of the participants. The study was conducted in India over 10 month.	B	Good

		allergy, heart disease, severe renal disease , epilepsy, hypertension and HB concentration < 8 g/dl.		additional oxytocic and side effects.			
Verma 2006	A double blind randomised controlled trial. The randomisation and concealment were not clearly described.	Participants were not described.	400 mcg sublingual misoprostol or methyl ergometrine 200 mcg IM.	The outcomes were mean blood loss, mean difference in haemoglobin, number of patients with total blood loss, additional use of oxytocics, manual removal of placenta, nausea, shivering and temperature ≥ 38 °C.	Intention to treat analysis with no loss of any of the participants . The study was conducted in India.	B	Poor Method of data collection was not reported
Vimala 2004	Randomised controlled trial. Randomisation was done by opening sequentially numbered sealed envelopes prepared using random number table.	Pregnant women at 37 weeks of gestation with spontaneous onset of labour. Exclusion criteria were oxytocin induction or augmentation of labour, caesarean delivery, grand multipara (parity >5), gestation < 37 weeks, multiple gestation, pregnancy induced hypertension, Hb concentration < 8g/dl and known hypersensitivity	400 mcg sublingual misoprostol or 200 mcg of IV injection methyl ergometrine .	The primary outcome was the drop in haemoglobin concentration . The secondary outcomes were estimated blood loss, additional use of uterotonics, duration of the third stage of labour, manual removal of placenta, side effects including	Intention to treat analysis with no loss of any of the participants . Study was done in India over 4 months.	A	Good

		to prostaglandins.		nausea, vomiting, giddiness, headache, shivering and elevated temperature >38 °C.			
Vimala 2006	Opened labelled randomised trial using computer generated random number in sealed opaque envelops.	Women at term (37-40 weeks) gestation scheduled for either elective or emergency lower segment caesarean section under regional anaesthesia. Exclusion criteria: any women with risks to PPH such as anaemia (Hb <8g%), multiple gestation, APH, polyhydraminos, prolonged labour, (>12 hours), two or more previous caesarean sections, history of rupture uterus, current or previous history of heart disease, liver, renal disorders or known coagulopathy.	400 mcg sublingual misoprostol and the control group received 20 IU oxytocin as IV infusion.	The primary outcomes are changes in haemoglobin levels after delivery, estimated amount of blood loss and the need for additional uterotonics therapy. The secondary outcomes are the incidence of side effects, need for blood transfusion and any other major complication.	Intention to treat analysis with no loss of any of the participants . Study was done in India over 9 months.	A	Poor Definition of fever and method of data collection were not reported
Walley 2000	Double blind placebo controlled trial using computer generated random numbers and numbered opaque packet. Containing a card indicating group allocation.	All women except, those who have risk factors for postpartum haemorrhage: grand multipara>5, multiple gestation, gestation< 32 weeks, gestational hypertension with	400 mcg oral misoprostol or 10 IU oxytocin IM.	Primary outcome drop in haemoglobin concentration . Side effects, nausea, vomiting, diarrhoea, shivering and elevated	Intention to treat analysis with less than 2% loss of participants to follow up. The study was conducted in Ghana	A	Good

		HELLP syndrome, hydramnios, previous postpartum haemorrhage, caesarean delivery, coagulation abnormalities, precipitatous labour (<3 hours), Chorioamnionitis and oxytocin induction or augmentation, women with hypersensitivity to prostaglandins, Hb concentration <8 g/dl.		temperature.	over 1 year and 3 months.		
Zachariah 2006	Randomised controlled trial using randomisation 1 of the 3 groups using computer-generated random numbers.	All women with SVD. Exclusion criteria: bronchial asthma, cardiac disease, rhesus factor incompatibility, pregnancy induced hypertension, caesarean delivery.	400 mcg oral misoprostol or 10 IU oxytocin, IM or 2 mg of ergometrine IV.	Primary outcomes: mount of blood loss>500 ml and 1000 ml, need for additional oxytocic. Secondary outcomes: duration of the third stage of labour, need for blood transfusion, haematocrit fall greater than>10%, adverse drug reactions such as pyrexia and shivering.	Intention to treat analysis with no loss of any of the participants . The study was conducted in India over 8 months. There was no report on method of concealment.	B	Good

Appendix A. 3. References of the included studies in the misoprostol induced fever review

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Appendix B. 1. Patient information sheet

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Participant's information sheet for the study of

Misoprostol to prevent bleeding after childbirth

You are being invited to take part in a research study. Before you decide whether to take part, it is important to understand why the research is being done and what it will involve. Please take time to read this information carefully, and ask us if you would like more information. You should only agree to take part if you want to – don't feel pressurised into taking part!

What is the purpose of the study?

Bleeding after childbirth is a major problem in the world, but especially in developing countries. The chance of bleeding after labour is about 1 in 10, but is halved by the use of preventive drugs. These are usually given to all women just after childbirth. Misoprostol is a drug that prevents bleeding after birth by making the uterus contract strongly. It is cheap, stable in hot climates and can be given orally (rather than needing an injection like the alternatives). For most people the treatment is effective and produces little or no side effects. However some women have side effects such as increased body temperature, diarrhoea or abdominal cramps. These are more common at high doses.

The best dose of misoprostol is not known. Although high doses might be more effective, they have higher rates of side effects. We therefore want to compare different doses to find the lowest effective dose of misoprostol.

Who is organising this research?

The research is being organised by the University of Liverpool and Misurata Teaching Hospital in Libya.

Why have I been chosen?

You have been chosen for the study because you are a healthy woman with a low risk of bleeding after labour (we are excluding any woman who has pregnancy complications or who has bled after previous deliveries or who has had a caesarean section). Your entry into the study will be confirmed after you give birth to make sure that you are still fit and well, and happy to take part.

Do I have to take part?

This is a voluntary study, and if you would prefer not to take part your decision will be accepted without question and will not affect the standard of care you receive. You are free to withdraw from the study at any time without giving a reason.

What will happen to me if I take part?

If you decide to take part in this study, you will receive the usual care ordered by your doctor. At delivery you will be given the study treatment which could be misoprostol, oxytocin or no treatment. The allocation of treatment will be random. After the delivery of the placenta, the study will be conducted by the researcher who is a University of Liverpool post graduation student and has a considerable amount of training in obstetrics and gynaecology in Libya. The researcher will introduce a small catheter connected to a monitor to your uterus to measure the pressure inside the uterus before the treatment. For the 2 hours observation in the labour ward you will be closely monitored by the investigator who will record your blood pressure, temperature and pulse and measure the pressure of the uterus using a computer programme. Also, you will be closely observed for the amount of blood loss. During this time you will just stay in bed for the 2 hours of the routine observation at the labour word and nothing will be required from you. At the end of the two hours observation you will be sent to the postnatal word and the treating staff will continue the observation for any unusual blood loss.

Are there any risks in taking part?

As with any woman, you might develop bleeding after delivery (this happens to around 7 in 100 women despite preventive treatment). This rate is about doubled in

women not receiving any preventative treatment. If you are in the group that will not receive any preventive drugs, then you will be observed closely and the same preventative drugs will be given to you at the first sign of any bleeding. Drugs for treatment of haemorrhage will be available to all women throughout the study in case they need them.

There may be some minor but short-lasting discomfort from having frequent monitoring of your blood pressure or from introducing the catheter. There are no side effects reported after using this catheter in the similar studies.

Misoprostol may cause diarrhoea which resolves spontaneously within 24 hours, abdominal cramps which can be managed with analgesics and misoprostol may also cause transient increase in body temperature which can be managed with paracetamol and cold sponges.

Are there any benefits in taking part?

Women taking part in this study will have a specialist obstetrician with the throughout their time in the study. This may be helpful to you if any complications develop.

The results of this study will not be of a direct benefit to you; however, the treatment given is meant to prevent bleeding after you giving birth and you will be closely observed and treated for any complications after delivery or any side effects of medications. The finding of this study may benefit patients using this treatment in the future.

What if I am unhappy or if there is a problem?

If you are unhappy, or if there is a problem, please feel free to let us know as we with you all the time from delivery till you transferred to the postnatal ward. Still if you have any problem you can contact the researcher Dr Anisa Elati or Prof. Mohammed Elmahaishi.

Will my taking part in this study be kept confidential?

All information collected about you during the course of the research will be kept strictly confidential and will not be disclosed to anyone. Any information about you, which leaves the research centres taking part, will have your name and address removed so that you cannot be recognized from it.

What will happen to the results of the research study?

Results of the project will be published in leading international journals.

What will happen if I want to stop taking part?

You can withdraw at any time without explanation and this will not affect the standard care you receive from your treating staff. Any information or data before withdrawal will be destroyed and no further use is made of them.

Who can I contact for further questions?

We can be reached on the following points of contact

Prof. Mohammed. S. Elmahaishi	Phone number,
Email address:	elmahaishi@elmahaishi.com
Dr Anisa Elati	Phone number,
Email address	elati@liv.ac.uk

مذكرة معلومات للمشاركات فى دراسة عن

عقار الميزوبرستول لمنع النزيف بعد الولادة

لقد تم دعوتك للمشاركة فى هذه الدراسة وقبل ان تتخذى قرارك بالمشاركة من المهم أن تعرفى لماذا تم إجراء هذا البحث. أرجو ان تأخدى وقتك فى قراءة هذه المعلومات جيدا وان تسألى الباحثة إذا رغبت فى معلومات أكثر. يجب أن توافقى على المشاركة إذا رغبت فى ذلك- لاتشعرى أنك مرغمة على المشاركة !

ما هو الغرض من هذه الدراسة؟

النزيف بعد الولادة من المشاكل الكبيرة فى العالم وخاصة فى دول العالم الثالث. معدل حدوث النزيف بعد الولادة حوالى 1 من 10 حالات ولادة ولكن يم انقاص هذا المعدل بمقدار النصف بإستخدام الدواء اثناء ولادة الجنين. ,عقار الميوبرستول هو أحد هذه الادوية التى تمنع النزيف بعد الولادة وذلك بجعل الرحم ينقبض بقوة. هذا الدواء رخيص ويحتمل درجات الحرارة فى الطقس الحار كما أنه يعطى عن طريق الفم (بدلاً من الحاجة إلى الحقن مثل بعض العقاقير الأخرى). لمعظم الناس هذا العلاج فعال وقد يسبب او لايسبب بعض الأعراض الجانبية. إلا أن بعض النساء قد تعانى من بعض الأعراض الجانبية مثل زيادة حرارة الجسم وإسهال أو مغص. وهذه الأعراض الجانبية تحدث مع أستعمال الجرعات الكبيرة.

الجرعة المثالية من الميزوبرستول غير معروفة. إلا أن الجرعات الكبيرة ذات تأثير أكبر ولها معدلات أكبر من الأعراض الجانبية. لهذا نحن نريد فى هذه الدراسة مقارنة جرعات مختلفة من هذا الدواء لإيجاد أقل جرعة فعالة من الميزوبرستول.

من هم منظمو البحث؟

هذا البحث تحت إشراف وتنظيم جامعة ليفربول فى بريطانيا ومستشفى مصراتة فى ليبيا.

لماذا تم اختيارك؟

لقد تم اختيارك لهذه الدراسة لأنك بصحة جيدة واقل عرضة لحدوث النزيف بعد اولادة (لقد تم استبعاد أى إمرأة لديها مضاعفات أثناء الحمل أو أى مريضة وضعت بعملية قصيرية أو سبق

وأن حدث لها نزيف فى ولادة سابقة). مشاركتك فى هذه الدراسة ستقرر بعد الولاة للتأكد من أنك يمكنك المشاركة وانك مازلت راغبة فى المشاركة.

هل يجب المشاركة فى الدراسة؟

لا، هذه الدراسة طوعية وإذا كنت لاترغبين فى المشاركة فإن قرارك سيقبل دون أى سؤال وهذا لن يؤثر على رعايتك وعلاجك المعتاد. وحتى بعد بدء الدراسة أنت حرة فى الإنسحاب من الدراسة فى أى وقت دون إ عطاء سبب لذلك.

ماذا سيحدث إذا شاركت فى الدراسة؟

إذا قررت المشاركة فى هذه الدراسة فإنك سوف تنالين الرعاية المعتادة والمصوفة لك من طبيبك. أثناء الولادة سوف تقدم لك أحد هذه الأختيارات. يمكن أن تكون ميزوبرستول أو أوكسيتوسين أو بدون دواء.العلاج سيتم تقريره بطريقة عشوائية. بعد خروج المشيمة، ستقوم الباحثة بإدخال أنبوب صغير إلى الرحم لقياس الضغط داخل الرحم لمدة ساعتين بعد الولادة. وستكونين تحت الملاحظة الدقيقة من قبل الباحثة وسيتم قياس ضغط الدمو الحرارة والنبض كل نصف ساعة. وسيتم مراقبة النزيف بعد الولادة بشكل دقيق. أثناء فترة المراقبة ستبقين فى السرير ولا تحتاجين لعمل أى شئ. وفى نهاية فترة ساعتى الملاحظة سوف تذهبين إلى قسم مابعد الولادة وسيستمر الطاقم الطبى هناك فى الملاحظة المستمرة ومراقبة اى نزيف غير عادى.

الباحثة هى الدكتورة أنيسة العاتى. وهى طبيبة تدربت ى امراض النساء والولادة عدة سنوات فى ليبيا والآن تقوم بإجراء أبحاث فى جامعة ليفربول فى المملكة المتحدة.

هل هناك أى خطورة من المشاركة فى الدراسة؟

كما يحدث لأى أمراة، يمكن أن يحدث لك نزيف بعد الولادة (هذا يحدث فى حوالى 7 من 100 أمراة بالرغم من العلاجات الواقية من النزيف). هذا المعدل قد يتضاعف فى النساء اللاتى لم تعطى هذا الدواء الواقى من النزيف. إذا كنت فى المجموعة التى لن تعطى علاج فإنه سوف يتم مراقبتك بشكل دقيق وسيكون العلاج متوفر فى حالة ظهور أول عرض للنزيف. الأدوية التى تستخدم لعلاج النزيف ستكون متوفرة لجميع النساء فى حالة حدوث أى نزيف.

من الممكن أن تكون هناك بعض التضايق البسيط من المراقبة المستمرة لضغط الدم ومن إدخال الانبوب إلى الرحم. لايوجد أى أعراض جانبية تم تسجيلها سابقاً فى دراسات مماثلة.

الميزوبرستول ممكن ان يسبب اسهال (وهو يتوقف بشكل تلقائى خلال 24 ساعة) ويمكن أن يسبب مغص فى المعدة (وهذا ممكن أن يعالج بالمسكنات البسيطة) وأيضا ممكن أن يسبب أزدياد فى درجة الحرارة (وهذا يمكن أن يعالج بالباراسيتامول و الكمادات الباردة).

هل هناك أى فائدة من المشاركة؟

إذا قررت المشاركة فى هذه الدراسة فإنك ستكونين تحت رعاية طبيب أخصائي طيلة فترة الدراسة. وهذا سيكون مهم فى حالة ظهور أي مضاغفات.

نتيجة هذه الدراسة سوف لن يكون لها أى منفغة مباشرة لك. إلا أن العلاج الذى أعطى لك يقصد به منع النزيف بعد اولادة وانت ستكونيين تحت الملاحظة وتتلقين العلاج فى حالة ظهور أية مضاعفات أوتاثيرات جانبية. نتيجة هذه الدراسة ربما تكون ذات نفع لأمهات أخريات فى المستقبل.

ماذا إذا كنت غير راضية أو هناك مشكلة؟

إذا كنت غير راضية أو هناك مشكلة، نرجو منك ان تخبيرينا بها فوراً. سنكون معك طوال الوقت من لحظة الولادة وحتى نقللك إلى قسم مابعد الولادة. إذا حدثت أى مشكلة بعد ذلك يمكنك الإتصال بالباحثة د/ أنيسة العاتى أو البروفيسور محمد المحيشى.

هل ستبقى مشاركتى فى الدراسة سرية؟

كل المعلومات التى سيتم جمعها عنك فى فترة الدراسة ستبقى فى غاية السرية وسوف لن تفصح لأحد. أى معلومات عنك تترك المستشفى سوف لن يكون عليها أسمك أو عنوانك.

ماذا سيحدث لنتائج هذا البحث؟

نتائج هذا البحث سوف تنشر فى أحد المجلات العلمية الرائدة.

ماذا سيحدث إذا قررت التوقف عن المشاركة؟

يمكنك الإنسحاب من المشاركة فى الدراسة فى اى وقت تشائين دون توضيح الأسباب وهذا لن يؤثر على مستوى الرعاية الذى تتلقينه. اى علومات أو بيانات عنك سوف نتخلص منها ولن تستخدم فى المستقبل.

بمن يمكننى الإتصال للحصول على معلومات أكثر؟

يمكنك الإتصال ب

البروفسور محمد المحيشى على الرقم 0912124313

د/ أنيسة العاتى 0917420612

Appendix B. 2. Consent form

Patient Identification Number for this trial:

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CONSENT FORM

Title of Project: Misoprostol for preventing postpartum haemorrhage

Name of Researchers: Dr. Anisa Elati

Please initial box

1. I confirm that I have read and understand the information sheet dated (May 2009) (version.3) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from The University of Liverpool. I give permission for these individuals to have access to my records. ☐

4. I am interested in taking part in the above study. ☐

Name of Patient	Date	Signature
Name of Person taking consent	Date	Signature

1 copy for the participant, 1 copy for the investigators, 1 copy for the case notes

UNIVERSITY OF LIVERPOOL

رقم المريض المشارك فى هذه الدراسة

نموذج الموافقة

عنوان الدراسة: الميزوبرستول لمنع النزيف بعد الولادة

اسم الباحثة: د. أنيسة العاتى

1. أوكد انى قرأت وفهمت مذكرة المعلومات المؤرخة (يوليو 2009) الإصدار 4.6 للدراسة المذكورة أعلاه
وأعطيت الفرصة لأفهم المعلومات واسأل الأسئلة وحصلت على إجابات وافية ☐

2. فهمت ان مشاركتى فى الدراسة طوعية وأننى املك كامل الحرية للإنسحاب من الدراسة فى أى وقت بدون إعطاء الأسباب وبدون أن يوثر ذلك على الرعاية الطبية لى ولايؤثر على حقوقى القانوننية ☐

3. فهمت أن اجزاء ذات العلاقة بموضوع الدراسة من بيانات ومعلومات طبية جمعت أثناء هذه الدراسة سيطلع عليها افراد من جامعة ليفربول. وأنا اسمح لهم بالإطلاع على سجلاتى. ☐

4. أقر انى وافقت على المشاركة فى الدراسة المذكورة أعلاه ☐

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أسم المريضة	التاريخ	التوقيع
----------	----------	----------
أسم الشخص الذى أخذ الموافقة	التاريخ	التوقيع

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Appendix B. 3. Case report for

Optimising the dose of Misoprostol for the prevention of postpartum haemorrhage: a randomised trial

Case Report Form

***On admission to the labour word (The** initial approach of participants)*

Women will be initially chosen for the study if

- *They have not had history of PPH, APH in this pregnancy.....* ☐
- *They have not had history of caesarean section.....* ☐
- *They have not had PROM in this pregnancy.....* ☐
- *They have not had Pre eclampsia in this pregnancy.....* ☐
- *They have not had history of infection in this pregnancy.....* ☐
- *They have not had history of polyhydraminous in this pregnancy.....* ☐
- *They are not anaemic (Hb >10 g/dl).....* ☐
- *They have singleton baby with gestational age > 34 weeks.....* ☐
- *They have 5 or less previous deliveries at over 28 weeks.....* ☐

Women should be chosen finally for the study if

- *They give informed written consent.....* ☐
- *They were not induced or augmented with any uterotonic drugs.....* ☐
- *They delivered SVD.....* ☐
- *They delivered a baby of < 4.00 kg.....* ☐

A) Background information

Patients' hospital Number ☐☐☐☐☐☐☐☐

Patient's name .....

Date of Birth ☐☐ /☐☐ / ☐☐ (dd/mm/yy)

OR Age ☐☐ Years

Date of Admission ☐☐ /☐☐ / ☐☐ (dd/mm/yy)

Time of Admission ☐☐ ☐☐ (24 hrs clock)

Consent for the study Yes ☐ No ☐

Past obstetrics history

No of deliveries beyond 24 weeks (exclude the one just delivered) ☐☐

This Pregnancy:

LMP ☐☐ /☐☐ / ☐☐ (dd/mm/yy)

Gestational age ☐☐ Weeks

Ante- partum haemoglobin level ☐☐. ☐ g/dl

B) Delivery

(Please, do not give oxytocin, syntometrine, methergine after the delivery of the baby)

Date of delivery ☐☐ / ☐☐ / ☐☐ (dd/mm/yy)

Time of delivery ☐☐ : ☐☐ (24 hrs clock)

Birth weight ☐☐☐☐ grams

Time of delivery of the placenta ☐☐ : ☐☐ (24 hrs clock)

Vital signs (after delivery of placenta)

Pulse ☐☐☐ beats per minutes **BP:** Systolic ☐☐☐ Diastolic ☐☐☐

Temperature (˚C) ☐☐.☐

C) Intrauterine pressure measurements

Insertion of the IUP catheter (Should be with in 1 minute of the delivery of the placenta)

Time of insertion ☐☐ : ☐☐ (24 hrs clock)

Continuous measurement of IUP starts immediately after it is inserted in the uterus (Data is saved in the computer)

Study treatment (envelop No).....

(Write the patient No on the envelope and keep (stable) with this form).

Time of treatment ☐☐ : ☐☐ (24 hrs clock)

Vital signs at (1) minutes

Pulse ☐☐☐ eats per minutes BP: Systolic ☐☐☐ Diastolic ☐☐☐

Temperature (C) ☐☐.☐

Vital sign at 30 minutes

Pulse ☐☐☐ beats / minutes BP: Systolic ☐☐☐ Diastolic ☐☐☐

Temperature (C) ☐☐.☐

Vital signs at 60 minutes

Pulse ☐☐☐ beats / minutes BP: Systolic ☐☐☐ Diastolic ☐☐☐

Temperature (C) ☐☐.☐

Vital signs at 90 minutes

Pulse ☐☐☐ beats per minutes BP: Systolic ☐☐☐ Diastolic ☐☐☐

Temperature (°C) ☐☐.☐

Vital signs at 120 minutes

Pulse ☐☐☐ beats per minutes BP: Systolic ☐☐☐ Diastolic ☐☐☐

Temperature (°C) ☐☐.☐

Blood loss from the time of the insertion of IUP until return to the post natal word

Unusual blood loss *Yes* ☐ *No* ☐ ☐☐☐☐ *ml*

Additional use of other uterotonic drugs to treat patients Yes ☐ No ☐

Treatment given..... Dose.....

D) ***Day after delivery***

Post partum haemoglobin level ☐☐.☐ *g/dl (24 hrs after delivery)*

When was the test taken? ☐☐ ☐☐ ☐☐ ☐☐ ☐☐

(d d / m m / y y) ***(24 hrs clock)***

Please ask the woman if she experienced any side effects after treatment

.....

Was there any maternal temperature in the first 24 hours? Yes ☐ *No* ☐

Note: *maternal temperature is defined as two successive reading of above 37.5°C taken between 1 and 12 hours apart, or one reading of over 38°C.*

Has the patient required blood transfusion since delivery? Yes ☐ *No* ☐

If yes, how many unites of blood? ☐☐ *Unites*

Study treatment given.....Dose.....Route.....

Appendix C. 1. Patient information sheet and consent form

A study of the side effects of misoprostol: a drug to treat hemorrhage after birth

Bleeding after childbirth occurs in about 1 in 10 women, and can be serious. It is usually treated by a drug called oxytocin which is given as an infusion into your arm. Recently another drug (misoprostol) has been found to be just as effective as oxytocin. Furthermore, it can be taken as tablets placed under the tongue and does not need to be kept cold. However, a raised body temperature is seen more commonly with misoprostol, affecting about a quarter of those who take it.

Ideally we would like to use a lower dose of the drug to reduce the rate of fever. The previous study used 4 tablets under the tongue – we want to use 3 instead and compare the effect on bleeding and raised body temperature. The study is being conducted at this hospital - Hospital Gineco-Obstétrico Isidro Ayora – in Quito, Ecuador. Samples will also be sent to the United Kingdom to test for the possibility of genetic reasons of fever after treatment with misoprostol.

> *If you agree to take part and develop very heavy bleeding after delivery, you will be given three pills to hold under your tongue to stop the bleeding. Women taking this medicine may experience nausea, chills, fever, vomiting, and diarrhoea. These symptoms occur in about in 1 in 10 people taking the drug, are usually not serious and go away in a short time. If you continue to bleed you will be given other drugs to stop the bleeding, as we would usually do.*

If you decide to participate, we will ask you some short questions. You will be also asked for a blood sample (about two spoons full) of blood drawn from your arm. This blood will be stored at this hospital and then sent to the University of Liverpool in The United Kingdom to be studied. The samples will be identified only by a code number, and it will not be possible to link you with any results from the blood in the

future. Your blood sample will be considered a donation to the University of Liverpool, and you will not be able to withdraw your blood sample after it has been assigned a code number and made not traceable to you. Your blood sample may be used in the future for other approved research by doctors at the University of Liverpool, but this cannot be traced back to you. The blood sample will not be used for commercial purposes.

If you are treated for heavy bleeding, a study nurse will ask you some questions about how you are feeling before you leave the hospital. We will also take another blood sample from your finger at this time. We will periodically take your temperature to evaluate whether or not you are experiencing a fever. If you have a fever, a study nurse will provide medication to lower your body temperature and make you feel more comfortable. You may also be contacted by study staff one-week following your discharge.

There are some benefits to participating in this study. Since you will have your blood taken, we will be able to give you information about you health, for example, if you have anemia. You will also have your blood loss monitored closely. One risk to participating in this study is the possible discomfort from the finger prick used to take blood. There is also a small chance of bruising and even smaller chance of infection at the site of the blood draw.

Any information you provide during the study will be confidential. Your name will not appear on any study document. Your participation is voluntary and you are free to withdraw from the study at any point. If you decide not to participate, you will receive standard care and treatment at this hospital.

Do you have any questions about the study?

Are you willing to participate?

Informed Consent, Part 2: Form for participants to sign

Assessment and pharmacogenetics of fever after misoprostol administration for the treatment of primary postpartum haemorrhage

Woman's
Name:.....

I have (been) read the information sheet concerning this study and I understand what is being asked of me if I participate in the study. All my questions and concerns have been addressed to my satisfaction. I understand that I can withdraw from the study at any time I wish without giving a reason and this will not affect the normal health care I receive.

I agree to take part in this study.

Woman'ssignature/thumb
print.....

Date.....

Study staff person Signature.....

If you have any questions regarding your participation in this study, please contact:

[Insert name and phone number of study staff person / PI]

Appendix C. 2. Form 6: blood sample / case review form

Woman's initials: _____ Study staff initials: _____

Study ID number: ___-___-___-___ Today's date: day____/ month____/ year____

I. Eligibility & Consent

1. Did give informed consent and sign form? ☐ 0 = No ☐ 1 = Yes
2. Was the woman diagnosed with PPH and given misoprostol treatment? ☐ 0 = No ☐ 1 = Yes
3. Was a blood sample taken? ☐ 0 = No ☐ 1 = Yes

II. Participant's information

4. Woman's age: ___ ___
5. Number of live births (include this one): _____
6. Self-reported ethnicity:

   Add categories per Ecuador census…
7. Is the woman currently taking any medication?
   ☐ 0 = No ☐ 1 = Yes

   If yes, please describe_____
   _____
8. Does the woman have any chronic illness?
   ☐ 0 = No ☐ 1 = Yes

   If yes, please describe_____
   _____
9. Woman's height: _____ cm
10. Woman's weight: _____ kg

III. Blood draw

11. Date/time misoprostol administered:

    Day___ Month___ Year____ / ___ ___:___ ___ hrs
12. Date/time of blood draw:

    Day___ Month___ Year____ / ___ ___:___ ___ hrs
13. Any problems collecting blood sample?
    ☐ 0 = No ☐ 1 = Yes

    If yes, please describe_____
    _____

IV. Recorded body temperatures

14. Baseline temperature pre-delivery: ___ ___.___ C
15. Temp 60 minutes post-treatment: ___ ___.___ C
16. Temp 90 minutes post-treatment: ___ ___.___ C
17. Did the woman experience fever ≥ 40.0 C at any time following treatment with misoprostol?
    ☐ 0 = No ☐ 1 = Yes

    If yes, please specify date/time:
    Day____ Mo____ Year ____ / ___ ___:___ ___ hrs

V. Side effects experienced post-treatment

18.	Nausea	☐ 0 = No	☐ 1 = Yes
19.	Vomiting	☐ 0 = No	☐ 1 = Yes
20.	Diarrhea	☐ 0 = No	☐ 1 = Yes
21.	Shivering/chills	☐ 0 = No	☐ 1 = Yes
22.	Fever	☐ 0 = No	☐ 1 = Yes
23.	Fainting	☐ 0 = No	☐ 1 = Yes
24.	Other	☐ 0 = No	☐ 1 = Yes

If yes, specify: _____

25. Additional comments:

Signature of Principal Investigator who reviewed/completed this form:
_____

Appendix C. 3. Patient information sheet and Consent form

UNIVERSITY OF LIVERPOOL

Liverpool Women's NHS
NHS Foundation Trust

Participant Information Sheet (version 2.1)

Project Title:

Pharmacogenetics of Misoprostol induced fever

What is the purpose of the study using your blood sample?

Misoprostol is widely used nowadays to treat many obstetrics and gynaecological problems and for most people the treatment is effective and produces little or no side effects. However, in some cases misoprostol can cause an increase in the body temperature, which resolves quickly. We do not know why some people develop high body temperature while others do not. The purpose of the study is to look for genetic markers from your blood that may be related to the increased temperature. In the future, this will hopefully allow us to develop a test that can help predict which patients are more susceptible to side effects and therefore develop better treatment strategies.

Who is organising this research?

The research is being organized by The University of Liverpool.

Why Have I been chosen?

You have been chosen for the study because you are going to have misoprostol as part of your treatment.

Do I have to take part?

This is a voluntary project, and if, when you have heard about the study, you would prefer not to take part; your decision will be accepted without question and will not

affect the standard of care you receive. You are free to withdraw from the study at any time without giving a reason.

What will happen to me if I take part?

If you decide to take part in this study, you will receive the usual clinical care ordered by your

doctor. You will be also asked to donate one sample of 9ml (equivalent to 2 tablespoons) of blood. Also, you will have your temperature measured before treatment and then at 1, 2 and 4 hours after having your treatment.

What if something goes wrong?

Taking your blood sample for the study has mild risk of pain and discomfort which is not expected to cause any kind of serious complication. After taking your blood sample, you will stay as routinely done by the department as a day case and you will be under close observation by the staff nurses. If you have a concern about any aspect of the study, you can speak to one of the researchers who will do their best to answer your questions.

What will happen to my blood test?

The Blood sample will be stored in this hospital and then sent to the Department of Pharmacology & Therapeutics at the University of Liverpool. All samples will be identified only by a code number, and so it will not be possible in the future to link you with any results from the blood sample. Your blood samples will be considered to be a gift to the University of Liverpool, which will act as custodian of all the samples obtained as part of this project.

What are the possible disadvantages and risks of taking part?

There may be some minor but short-lasting discomfort from having a blood test. Taking part in the study will not affect your current treatment, nor will it affect your ability to obtain insurance for health purposes.

What are the possible benefits of taking part?

The studies will not be of a direct benefit to you; however, it may benefit patients using this treatment in the future.

Will my taking part in this study be kept confidential?

As stated above, your samples will be anonymised, and the genetic information obtained from the DNA sample will be kept strictly confidential and not be disclosed to anyone. All information collected about you during the course of the research will be kept strictly confidential. Any information about you, which leaves the research centers, will have your name and address removed so that you cannot be recognized from it.

What will happen to the results of the research study?

Results of the project will be published in leading international journals.

Who has approved the study?

This research study has been reviewed and approved by Liverpool Adult Research Ethics Committee.

Who can I contact for further details or with questions?

We can be reached on the following points of contact.

Dr. Andrew Weeks	Tel: 0151 702 4240	email: aweeks@liverpool.ac.uk
Dr. Anisa Elati	Tel 01517024109	email: elati@liverpool.ac.uk
Miss. Kay Holland	Tel 0151 708 9988 Ext 1123/1130	email: kay.holland@lwh.nhs.uk

Thank you for considering this research.

Centre Number:

Study Number: Patient Identification Number for this trial:

CONSENT FORM

Title of Project: **Pharmacogenetics of Misoprostol Induced Fever**

Name of Researchers: Dr. Andrew Weeks Dr. Anisa Elati

Please initial box

1. I confirm that I have read and understand the information sheet dated March 2009 (version.2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from The University of Liverpool or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to take part in the above study. ☐

Name of Patient	Date	Signature
Name of Person taking consent	Date	Signature

Address of participant (only if she wants a copy of the study results)

.....

.....

1 copy for the participant, 1 copy for the investigators, 1 copy for the case notes

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

Appendix C. 4. Case Report Form

PMIF: The Pharmacogenetics of Misoprostol Induced Fever

Patients' Code Number ☐☐☐☐☐☐

Patient's name ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Date of Birth ☐☐ / ☐☐ / ☐☐ (dd/mm/yy)

Date of Admission ☐☐ / ☐☐ / ☐☐ (dd/mm/yy)

Time of Admission ☐☐ : ☐☐ (24 hrs clock)

Consent for the genetics study Yes ☐ ☐

1. Background

Obstetrics history No of times pregnant (including this pregnancy) ☐☐

No of pregnancy > 24 weeks ☐☐

Previous termination of pregnancy Yes ☐ ☐

If yes, what was the method of termination? Surgical ☐ Medical ☐

If medical, did you take misoprostol? Ye☐ No ☐

If yes, do you have any side effects with misoprostol Y☐ No ☐

If yes, what were the side effects? .....

Medical history

Any chronic illness

Yes ☐ No ☐

If **yes,**

Current medication

Drug name	Dose	Frequency

Gestational age (today) ☐☐ / ☐☐ eeks/ days

Patient's weight ☐☐☐ Kgs patient's height ☐☐☐ cm

Assessed by (circle): USS/ LMP/PV

2. Observation of temperature

Misoprostol received at ☐☐ : ☐☐ (24 hrs clock)

Temperature is ☐☐ ☐ °C at ☐☐ : ☐☐ (24 hrs clock)

	Tempreature (°C)		**Time taking temperature (24 hrs clock)**
2 hrs after misoprostol	☐☐ . ☐	at	☐☐ : ☐☐
4 hrs after misoprostol	☐☐ ☐	at	☐☐ : ☐☐
6 hrs after misoprostol	☐☐ . ☐	at	☐☐ : ☐☐

3. Side effects (Patient complaint) (please complete before discharge)

Coldness	yes		No	
Chills	yes		No	
Fever	yes		No	
Abdominal cramps	yes		No	
Nausea/Vomiting	Yes		No	
Diarrhoea	Yes		No	

Blood sample given Yes ☐ No ☐

CRF completed by

Name ..... signature..... Date.....

Patients self report of ethnicity

I would describe my ethnic origin as (please mark one box only)

White - White British		**Asian or Asian British** - Indian	
White Irish		Pakistani	
Other white background		Bangladeshi	
Mixed - White and Black Caribbean		Other Asian background	
White and Black African		**Black or Black British** - Black Caribbean	
White and Asian		Black African	
Other mixed background		Other black background	
		Chinese or other Chinese	
		ethnic group Other ethnic	
		back ground	

Appendix C. 5. Sequenom MassArray: iPLEX Protocol

- Reagents and Consumables
- PCR preparation
- Preparation and Addition of SAP Enzyme Solution
- Preparation and Addition of iPLEX Reaction Cocktail Solution
- Cleaning up the iPLEX Reaction Products
- Transfer of the iPLEX Reaction Products onto a SpectroCHIP
- Reading the SpectroCHIP in the MassARRAY READER RT instrument
- Analyzing the Data on the MassARRAY TYPER
- Equipment maintainance protocols

Reagents and Consumables

Plates & Seals:

Description	Supplier	Item #
384 well Plates, 50 plates	ABgene	TF-0384
Adhesive PCR- Film, 100 each	Abgene	AB-0558
96 well Sarstedt- plates, 100 plates	Sarstedt	82/1.583

PCR machine:

- G-Storm with 384 well blocks (located in Molecular Lab)

Pipettes:

- Two sets of single channel pipettes (e.g. Eppendorf with 2.5µl, 20µl, 200µl and 1000µl volume) one per PCR and one post PCR set.
- Multichannel pipettes (e.g. Eppendorf with 10µl and 50µl volume)
- Tips

Centrifuge:

- Plate centrifuge with at least 3000rpm

Other components:

- Water system capable of producing at least 18.2 MΩ/cm resistivity
- Reaction tubes (500µl – 2000µl)
- 30µl tips for the Matrix
- 100% Ethanol (for the nanodispenser)
- PCR reagents
    - Hot Star Taq (Qiagen)
    - dNTP mix
    - PCR & Extend Primer

- iPLEX Gold Kit (Sequenom) containing: - SAP- buffer

- SAP- enzyme

- iPLEX- Termination- mix

- iPLEX- buffer

- iPLEX- enzyme

- Resin (28g)

- 10 x 384 Spectro chips

Primers:

Primers (supplied by Metabion): PCR primer **50µM** each

Extend primer for 36 plex **400µM** each and for 24 plex **300µM** each

Protocol

Preparation of plex primer mix;

For 384-well plate:

- 36-plex: 5µl each Forward primer + 5µl each Reverse Primer + 140µl Nanopure water (500µl total volume)
- 24-plex: 5µl each Forward primer + 5µl each Reverse Primer + 260µl Nanopure water (500µl total volume)

PCR preparation:

- Prepare the primer mix for each plex to a working concentration of **0.5µM** each primer.
- Prepare the PCR mix according to Table 1 (volumes for a 384-well Microplate include 25% overhang to account for possible pipetting loss).
- For plexes >29 the PCR mix for 36-plex should be used- otherwise 24-plex mixed should used.

Table 1: PCR mix preparation

Reagent	**Volume for Single Rx**	**Volume for one 24-plex (384 well)**	**Volume for Single Rx**	**Volume for one 36-plex (384 well)**
- Nanopure Water	1.85 µl	888.00 µL	1.85 µl	840.00 µL
PCR Buffer (10x)	0.625 µl	300.00 µL	0.625 µl	300.00 µL
$MgCl_2$ (25mM)	0.325 µl	156.00 µL	0.325 µl	156.00 µL
dNTP mix (25mM)	0.10 µl	48.00 µL	0.10 µl	48.00 µL
primer mix (0.5µM)	1.00 µl	480.00 µL	1.00 µl	480.00 µL
Hot Star Taq	*0.10 µl*	48.00 µL	*0.20 µl*	96.00 µL

(5 U/µl)				
Total Volume	**4.00 µl**	**1920.00 µL**	**4.00 µl**	**1920.00 µL**
DNA (10 ng/µl)	1.0 µl	-	1.0 µl	-

- Add 4µl PCR mix to each well of the 384 well plate, add 1µl DNA to each well of the 384 well plate.

OR

- Add 97µl PCR cocktail to one row of a 96 well plate than add 24.25µl DNA. From this mix, dispense 5µl to a 384 well plate

Seal Microplate with Adhesive PCR Seal (AB-0558)

Cycling: Run G-Storm Thermocycler Program: C\ GStorm\ Users\ Guest\ Scripts

- Sequenom\ PCR IPLEX.scr

Reaction volume: 5µl

Approximate running time: 2hrs 30mins

Preparation and Addition of SAP Enzyme Solution

- Prepare the SAP Enzyme Solution according to Table 2 (volumes for a 384-well Microplate include 38% overhang to account for possible pipetting loss)
- Table 2: SAP Enzyme Solution Preparation

Reagent	**Volume for Single Rx**	**Volume for 384-Well MTP**
Nanopure Water	1.53 µl	810.78 µl
hME Buffer (10x)	0.17 µl	90.08 µl
Shrimp alkaline phosphatase (SAP)	0.30 µl	158.98 µl
Total Volume	**2.00 µl**	**1059.84 µl**

- In a new 96-well, polystyrene microplate, pipette **85 µl** of SAP enzyme solution into each well H01 – H12.
- Using a twelve-channel pipettor, draw from the wells in row H and distribute **10 µl** to each well in rows A-G.

Addition of SAP to 384-well plate using Matrix Liquid handler

- Open "ControlMate" software
- Select "Open Sequence"- select "maintenance methods"- select "Liquid Handler tip wash.cms"
- When prompted for password, click "Cancel"
- Select "Run" from toolbar
- Repeat "Open Sequence"- select folder "384 methods"- select "iPLE-hME Methods"- select "SAP addition (96 to 384).cms"
- Check plates are positioned as pop-up diagram shows and close to start- Note: 384-well plate should always be placed in metal holder provided.
- Seal the microplate with AB-0558

Cycling: Run G-Storm Thermocycler Program: C\ GStorm\ Users\ Guest\ Scripts

-Sequenom\ SAP IPLEX.scr

Reaction volume: 7μl

Approximate running time: 1hr

Preparation and Addition of iPLEX Gold Reaction Cocktail

Table 3: Preparation of Extend primer mix for 36-plex reaction using 4-step adjustment

Extend Primer Group	**Final Concentration/ primer**	**Volume/ primer**	**36-plex (9 primers) (total volume)**
1	7μM	8.75μl	78.75μl
2	9.3μM	11.63μl	104.63μl
3	11.66μM	14.58μl	131.22μl
4	14μM	17.5μl	157.5μl
Nanopure Water			27.9μl (to500ul)

For <29-plex: same volumes apply but stock primers should be at 300μM not 400μM

- Prepare the Extend primer mix using 4- Step adjustment.
- Prepare the iPLEX Reaction Cocktail Solution as described in Table 4 (volumes for a 384-well Microplate include 38% overhang to account for possible pipetting loss).
- Table 4: iPLEX Reaction Cocktail Solution Preparation

Reagent	Volume for Single Rx	Volume for one plex (384 wells)
- H2O	0.755 µl	400.10µl
iPLEX-Buffer (10x)	0.2 µl	105.98µl
iPLEX-Termination mix	0.2 µl	105.98µl
Primer mix	0.804 µl	426.06µl
iPLEX-Enzyme	0.04 µl	21.20µl
Total	**2.0 µl**	**1059.34µl**

- In a new 96-well, polystyrene microplate, pipette **85 µl** of iPLEX reaction cocktail solution into row H.
- Using a twelve-channel pipettor, draw from the wells in row H and distribute **10 µl** to each well in rows A-G.

Addition of iPLEX reaction mix to 384-well plate usingMatrix Liquid handler

- Open "ControlMate" software
- Select "Open Sequence"- select "384 methods"- select "iPLEX-hME Methods"- select "Cocktail addition (96-384).cms"
- When prompted for password, click "Cancel"
- Select "Run" from toolbar
- Check plates are positioned as pop-up diagram shows and close to start
- Seal the microplate with AB-0558

Cycling: Run G-Storm Thermocycler Program: C\ GStorm\ Users\ Guest\ Scripts

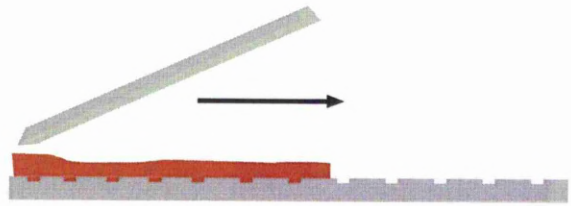
-Sequenom\ SAP IPLEX.scr

Reaction volume: 9µl

Approximate running time: 4hrs

Cleaning up the iPLEX Reaction Products

- Apply 2 smalls scoops of CLEAN resin to the left-hand side of the 6mg dispense 384-well dimple plate
- Using the beveled Perspex applicator spread the resin into the wells as indicated below:



- Any excess unused resin can be returned to the pot for future use
- Take the 384-well and invert. Place on directly on top of the resin dimple plate. Invert both and tap gently to allow the resin to drop into the wells of the thermoplate.
- Open "ControlMate" software
- Select "Open Sequence"- select "384 methods"- select "iPLEX-hME Methods"- select "16ul water addition.cms"
- When prompted for password, click "Cancel"
- Select "Run" from toolbar
- Check plates are positioned as pop-up diagram shows and close to start
- Seal the microplate with AB-0558
- Place the plate between 2 polystyrene blocks and secure in the Heidolph- Reax 2 rotator and set to level 1. Rotate plate for 10 min.
- Centrifuge the sample plate for 5 min at 3000 RPM.

Transfer of the iPLEX Reaction Products onto a SpectroCHIP

On the nanodispenser PC, open the program "Spectropoint"

- Log-in as User: "Op1" with password "Op1"
- Release emergency stop button on the nanodispenser.
- Select function "Home machine"
- Select function "Pin conditioning" and then "Drain Sonicator"
- Refill the sonicator bath with 100% ethanol using a squeezy bottle.
- Check the box marked "Main Head" and "Start" Run should take 30mins.
- Repeat process with "Single Head"- this can be stopped after 5mins.
- Select "Drain Sonicator" then "Fill Sonicator"
- Place the microtiter plate and an old SpectroCHIP on the deck of the MassARRAY® Nanodispenser S instrument.

- Before dispensing the Chip, run the volume check to avoid poor dispensing quality
- Under *Load Method* go to *System* change the file type from *.tmf to *.vmf, select the file *Volume384.vmf.*
- Run the volume check to adjust the dispense speed.
- In the *Run Setup tab* Load the file *iPLEX*, change the dispense speed to that speed which gave the best results in the volume check.
- Replace Chip with new one.
- Select the *Status* tab, click *Start.*
- Using the MassARRAY® Nanodispenser a few nanoliters (approx. 15 nl) of the samples are transferred onto a 384 SpectroCHIP.
- Add 70 µl calibrant to the calibrant reservoir on the MassARRAY® Nanodispenser S instrument.
- In the *Run Setup tab* Load the file *Calibrant dispense* and select the *Start* button in the *Status tab.*
- When complete, the nanodispenser can be shut down to a sleep mode by running the "Clean" function and pushing the red emergency stop button when the pins are in the sonicator bath.

Reading the SpectroCHIP in the MassARRAY® READER RT MALDI-TOF instrument

- On the RT computer make sure FLEXControl and ServerControl are running.
- Make sure the correct Method (iPLEX.par) and Sample Carrier (SequenomChip384C) are loaded in the FLEXControl.
- Make sure MassARRAY® CALLER is running.
- In the *Plate Editor* create a chip with assays and samples.
- In the MassARRAY Typer folder select *ChipLinker.*
- Connect to your database.
- Select the plate you want to analyze.
- Select iPLEX
- Under *Dispenser* select *Nanodispenser S.*
- Under *Process Method* select *Genotype.*
- Enter an *Experiment Name* (e.g. the chip name entered in the *Plate Editor*).

- Enter a Chip Barcode.
- Click *Add.*
- Click *Create.*
- Click *Done.*
- In SpectroAQUIRE in the *Auto Run Set Up* tab under *Barcodes for individual chips* enter the name of the SpectroCHIP plus experiment number you want to run (it is recommended do to so with the Copy Paste function) at the position the chip is loaded on the Scout Plate.
- Make sure that the Parameter file *iPLEX* is loaded.
- Place the Chip on Scout target.
- Introduce target in the MassARRAY® READER with the *In/Out* function on the Autoflex instrument.
    - In SpectroAQUIRE in the *Auto Run Set Up* tab switch on the high voltage.
- Click the *Auto Run* tab under *Run* select *Start Auto Run.*

Analyzing the Data on the MassARRAY® TYPER

- In the *Typer Analyzer* you get a table with the results, can look at the corresponding spectra and print out reports with the results and statistics.

Appendix C. 6. PCR primers, forward primers reversed primers &extension primers for the multiplexed assay.

No	Type	Oligo Name	Sequence 5'-3'
1	MTP-96	A-Plex 1 rs35646917	ACGTTGGATGTCACTGAATGCAGTTCATCC
2	MTP-96	A-Plex 1 rs3742106	ACGTTGGATGCAACCAAAATGTCAAGTCCG
3	MTP-96	A-Plex 1 rs2253170	ACGTTGGATGGTTTTCCTTCATGCACTTGG
4	MTP-96	A-Plex 1 rs1801253	ACGTTGGATGCCTTCAACCCCATCATCTAC
5	MTP-96	A-Plex 1 rs45439401	ACGTTGGATGTCTCAATCAGTATACACC
6	MTP-96	A-Plex 1 rs17197	ACGTTGGATGAAAGTGTCAGAAGGAGCTAC
7	MTP-96	A-Plex 1 rs4998	ACGTTGGATGAGCCTTGAACTTCACTCCTC
8	MTP-96	A-Plex 1 rs11045825	ACGTTGGATGAGGAATTTAGGGTGTGATGG
9	MTP-96	A-Plex 1 rs114509093	ACGTTGGATGAGTGGCAGCGGCGGTACTT
10	MTP-96	A-Plex 1 rs34550074	ACGTTGGATGATGATGGTGGTAGCTATGCG
11	MTP-96	A-Plex 1 rs4149087	ACGTTGGATGACAAACACAGAGTTTGAAC
12	MTP-96	A-Plex 1 rs2274407	ACGTTGGATGTATCTGGTTGACATCACTGC
13	MTP-96	A-Plex 1 rs114869610	ACGTTGGATGAGGGTTCATATGGACAGCAC
14	MTP-96	A-Plex 1 rs4148551	ACGTTGGATGACAACAACAAAAACCTGTG
15	MTP-96	A-Plex 1 rs4149085	ACGTTGGATGGGTGGAAGTATAAATAAGCC
16	MTP-96	A-Plex 1 rs708502	ACGTTGGATGATTAGGGCAGCTTCATTTTG
17	MTP-96	A-Plex 1 rs16870224	ACGTTGGATGGTCTCACTAAAGCATGAAATG
18	MTP-96	A-Plex 1 rs11045879	ACGTTGGATGGGATCCAGGGTTAATATAAC
19	MTP-96	A-Plex 2 rs1042714	ACGTTGGATGAGACATGACGATGCCCATGC
20	MTP-96	A-Plex 2 rs72978388	ACGTTGGATGCAGTGTTGAGTCCTCCATTG
21	MTP-96	A-Plex 2 rs16993929	ACGTTGGATGATGTGCAACGAGTTACTGCC
22	MTP-96	A-Plex 2 rs3819783	ACGTTGGATGTTCAACTAAACTTGCAGCTC
23	MTP-96	A-Plex 2 rs3765534	ACGTTGGATGTTCCTAAGTACCAGTTAAG
24	MTP-96	A-Plex 2 rs879894	ACGTTGGATGAGAGCGAGACTCCGTATGAA
25	MTP-96	A-Plex 2 rs45461592	ACGTTGGATGTTAATGAGAATTAACTGCC
26	MTP-96	A-Plex 2 rs76026776	ACGTTGGATGACAGAGAACCTGTATTAGGG
27	MTP-96	A-Plex 2 rs34671512	ACGTTGGATGGATATTTTTTCTTCATGGC
28	MTP-96	A-Plex 2 rs4148553	ACGTTGGATGATGGAAGCCCTTAAAGGTGC
29	MTP-96	A-Plex 2 rs211035	ACGTTGGATGTGTTTTGTTTTGGACAGGG
30	MTP-96	A-Plex 2 rs113569514	ACGTTGGATGGAGACTGAGCCAGCGAGTG
31	MTP-96	A-Plex 2 rs34559063	ACGTTGGATGGTGATCATAATGAGGTTTG
32	MTP-96	A-Plex 2 rs6439448	ACGTTGGATGTCAGTTACTATAAGGTGTCG
33	MTP-96	A-Plex 2 rs211014	ACGTTGGATGTAGCCTATCTGCAGGCTAAG

34	MTP-96	B-Plex 1 rs35646917	ACGTTGGATGAGAAGGGAAGGGAGTAAGTG
35	MTP-96	B-Plex 1 rs3742106	ACGTTGGATGCAATGTGGTTTACATAGTCC
36	MTP-96	B-Plex 1 rs2253170	ACGTTGGATGTAGTTCATAACTTTTTATCC
37	MTP-96	B-Plex 1 rs1801253	ACGTTGGATGAGCCCTGCGCGCGCAGCAGA
38	MTP-96	B-Plex 1 rs45439401	ACGTTGGATGTCTTACCTTCTATAGGTAGC
39	MTP-96	B-Plex 1 rs17197	ACGTTGGATGTCAATGCAGCCGATTGTTCC
40	MTP-96	B-Plex 1 rs4998	ACGTTGGATGGTGCCCCTACCAAAGCCAG
41	MTP-96	B-Plex 1 rs11045825	ACGTTGGATGAGTGCTTTTGGATGCATGGG
42	MTP-96	B-Plex 1 rs114509093	ACGTTGGATGCTCCTTACCGGGGCCTCGC
43	MTP-96	B-Plex 1 rs34550074	ACGTTGGATGGGATGCTGTTTGGAGGAATC
44	MTP-96	B-Plex 1 rs4149087	ACGTTGGATGGTGAGTAACAGATATTATTG
45	MTP-96	B-Plex 1 rs2274407	ACGTTGGATGGGAACTTCTCAGAATCTTGG
46	MTP-96	B-Plex 1 rs114869610	ACGTTGGATGATTACCTGCATGCAGGCACC
47	MTP-96	B-Plex 1 rs4148551	ACGTTGGATGTGTGCCTTAAGAGACTACAG
48	MTP-96	B-Plex 1 rs4149085	ACGTTGGATGTGTAACAATGAGTACTCTC
49	MTP-96	B-Plex 1 rs708502	ACGTTGGATGGCATCTGGAAGATCATTTTG
50	MTP-96	B-Plex 1 rs16870224	ACGTTGGATGCCTTCTCACAGAGAAGAAA
51	MTP-96	B-Plex 1 rs11045879	ACGTTGGATGCTTGATGGCTTCCAAGTTTC
52	MTP-96	B-Plex 2 rs1042714	ACGTTGGATGAAGCCATGCGCCGGACCA
53	MTP-96	B-Plex 2 rs72978388	ACGTTGGATGTCTCTGTTTAGGATTCCCCC
54	MTP-96	B-Plex 2 rs16993929	ACGTTGGATGTTACTGAGGGATGCAACCCG
55	MTP-96	B-Plex 2 rs3819783	ACGTTGGATGAAAGTTTGCTGGCAGATTGG
56	MTP-96	B-Plex 2 rs3765534	ACGTTGGATGGTCACTGTAAATGGAGGAGG
57	MTP-96	B-Plex 2 rs879894	ACGTTGGATGCCCACTAGGTTGGAGGTGA
58	MTP-96	B-Plex 2 rs45461592	ACGTTGGATGGTGATCAAGAAGACTTTAGG
59	MTP-96	B-Plex 2 rs76026776	ACGTTGGATGTGTTGCTATACATCCCAAAG
60	MTP-96	B-Plex 2 rs34671512	ACGTTGGATGCTTGGGCTTGTCTTCAATG
61	MTP-96	B-Plex 2 rs4148553	ACGTTGGATGACTCTAAAGGAGGATGGACG
62	MTP-96	B-Plex 2 rs211035	ACGTTGGATGGATCACACCACTGCACATTC
63	MTP-96	B-Plex 2 rs113569514	ACGTTGGATGGGAGAGCGCGTTTCATCATC
64	MTP-96	B-Plex 2 rs34559063	ACGTTGGATGGTCTTTAACCTCAAAAGTCC
65	MTP-96	B-Plex 2 rs6439448	ACGTTGGATGCCTGGGTAATGAGGCACAGT
66	MTP-96	B-Plex 2 rs211014	ACGTTGGATGTGGCCTGGCTAAACTCATAC
67	MTP-96	E-Plex 1 rs35646917	CAGTTCATCCTCGGCCC
68	MTP-96	E-Plex 1 rs3742106	CCGTTCCGAAGGCATTT
69	MTP-96	E-Plex 1 rs2253170	ATGCACTTGGTGATAAAC
70	MTP-96	E-Plex 1 rs1801253	CTTCCGCAAGGCCTTCCAG
71	MTP-96	E-Plex 1	AATCAGTATACACCAGAGAT

		rs45439401	
72	MTP-96	E-Plex 1 rs17197	tctCTACAAAACCTACCCTCA
73	MTP-96	E-Plex 1 rs4998	gCCCTCAGTGGTAGTGTCCAG
74	MTP-96	E-Plex 1 rs11045825	TGATGGTTAGAATAGGAGAAT
75	MTP-96	E-Plex 1 rs114509093	gagaGGCGGTACTTCTACCTGT
76	MTP-96	E-Plex 1 rs34550074	ggGGTAGCTATGCGGGGAATGG
77	MTP-96	E-Plex 1 rs4149087	ACACAGAGTTTGAACTATAATAC
78	MTP-96	E-Plex 1 rs2274407	ccacAAACCTGTACTCTCTTTCAG
79	MTP-96	E-Plex 1 rs114869610	tttaATATGGACAGCACTTAGATG
80	MTP-96	E-Plex 1 rs4148551	ccacCAACAAAAACCTGTGACAACT
81	MTP-96	E-Plex 1 rs4149085	AACTTATAATAAAACAAACTGTAGG
82	MTP-96	E-Plex 1 rs708502	actgACACACTGTTTTCATTTCTCCA
83	MTP-96	E-Plex 1 rs16870224	aTTTTATTGTTGTACATACGATTTAA
84	MTP-96	E-Plex 1 rs11045879	agcaAGGGTTAATATAACAGAATCAA
85	MTP-96	E-Plex 2 rs1042714	CCACACCTCGTCCCTTT
86	MTP-96	E-Plex 2 rs72978388	CTCCATTGAGGATGCCC
87	MTP-96	E-Plex 2 rs16993929	GTTACTGCCGATAATGAAA
88	MTP-96	E-Plex 2 rs3819783	gTTGCAGCTCTGCTGTGTTC
89	MTP-96	E-Plex 2 rs3765534	ACCAGTTAAGATCTAGCTTCT
90	MTP-96	E-Plex 2 rs879894	TTACTACGGAGAAATAAACAG
91	MTP-96	E-Plex 2 rs45461592	cctcATTAACTGCCTTGAGACA
92	MTP-96	E-Plex 2 rs76026776	gtagTAGGGGTCAGTCCTTGAA
93	MTP-96	E-Plex 2 rs34671512	gATTTTTTCTTCATGGCATAAAT
94	MTP-96	E-Plex 2 rs4148553	ggggAAGGTGCTTTGATACTGAA
95	MTP-96	E-Plex 2 rs211035	ttgtgTTGGACAGGGTCTCGTTCT
96	MTP-96	E-Plex 2 rs113569514	ccccCTGAGCCAGCGAGTGCGCGCC
97	MTP-96	E-Plex 2 rs34559063	GTGATCATAATGAGGTTTGTTAAAA
98	MTP-96	E-Plex 2 rs6439448	TTCTGACTATAAATCCTGAAATAATG
99	MTP-96	E-Plex 2 rs211014	ggatGGCTAAGGCTCAGCAGTTTGGG

N.B: A- and B- primers are the forward and reverse PCR primers, and E-primers are extension primers.

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Review article

The use of misoprostol in obstetrics and gynaecology

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Misoprostol, although originally introduced as a therapy for gastric ulcers, is now widely used in reproductive health. For some indications it is now the optimal choice, whilst for others it provides an important alternative, especially in low-resource settings. The optimal dose varies widely from 20 to 600 mcg depending on the indication and gestation. Use of the correct dose is important, too low a dose will be ineffective and overdosage can be dangerous for mother and baby. Evidence-based information about the safest regimens for multiple pregnancy indications are therefore provided in this review.

Keywords Misoprostol, low-resource settings, dose, mother, pregnancy, safest.

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Misoprostol (15-deoxy-16-hydroxy-16-methyl PGE1) is a stable, synthetic form of prostaglandin E1 analogue. It has anti-secretory and mucosal protective properties and was originally developed in the 1970s for the prevention of nonsteroidal anti-inflammatory drugs (NSAIDS)-induced peptic ulcers.[1] Its use for that indication however, has become limited and it is now used much more widely for 'off-label' indications in obstetrics and gynaecology.

Misoprostol has several properties that make it especially suitable for reducing maternal mortality in low-resource areas. The tablets are thermo-stable and have a long shelf life at room temperature whilst in aluminium blister packets. Misoprostol tablets could also be easily administered by unskilled attendants or the women themselves thus making them available for women giving birth at home or in isolated areas. This is in contrast to the alternative uterotonic drugs such as oxytocin and prostaglandins which are unstable at room temperature and require injection. Initially, misoprostol was only commercially available as an oral tablet in 100 and 200 μg strengths. However, the tablets are also effective when administered vaginally, rectally, bucally and sublingually. The rate of absorption varies considerably between routes and care must be taken to use the correct dose and frequency for the specified route.

The original pricing of the Cytotec® (Pfizer Inc, New York, NY, USA) brand of misoprostol was based on a 6-week course of 600 mcg daily for gastric ulcer treatment. This makes the small doses required for reproductive health indications very inexpensive, especially when compared with the alternatives for labour induction. It is, however, currently slightly more expensive than oxytocin (with syringe and needle) for the management of postpartum haemorrhage (PPH). Whilst the arrival of new low dose generic misoprostol tablets is welcome, the price is likely to be higher than for Cytotec®, and practitioners will need to balance the increased cost against the benefits in safety and logistics of having an accurately sized formulation.

The slow pace of misoprostol research along with the lack of progress in achieving the millennium development goal 5 has led to a call to 'fast track' the introduction of misoprostol without waiting for confirmatory trials.[2] On the premise that in emergencies, policies may be implemented based on evidence from good science, but not necessarily randomised controlled trials (RCTs), misoprostol has now been introduced for PPH management in a number of countries including Nigeria, Uganda, Ethiopia, Bangladesh, India, Nepal, Tanzania, and Zambia.

The ease with which misoprostol can be used to induce abortions has led to a very cautious approach to its introduction from both the manufacturer and national governments. And so, despite the large body of evidence supporting the beneficial effects of misoprostol on women's reproductive health, the manufacturers (Searle, now Pfizer) have strongly warned obstetricians and physicians that misoprostol should

not be used in pregnant women.[3] The original and most widely available preparation of misoprostol (Cytotec®) therefore remains off-label for reproductive health uses. Despite this, the efficacy and safety of misoprostol for termination of pregnancy (TOP) has now been acknowledged by the US Federal Drug Authority (FDA) by their inclusion of it into the mifepristone TOP regimen.[3] Furthermore, there is also an increasing recognition of the limitations of the drug licensing process in pregnancy. The lack of a licence does not show a lack of efficacy or safety, but rather demonstrates that it is not cost-effective for the pharmaceutical company to obtain a licence. This is common in high-risk indications (e.g. pregnancy) and with low cost generic drugs. Hence, some of the most important drugs in obstetrics (e.g. corticosteroids to promote fetal lung maturity and oxytocin 10 IU IM for PPH prophylaxis) remain off-label for pregnancy use. As if to demonstrate this point, misoprostol is now considered an 'essential drug' by the World Health Organisation (WHO) for the induction of labour, medical TOP, and incomplete abortion (Table 1).

The lack of a specific licence for Cytotec® has led to a number of problems. These include distrust on the part of national drug authorities, the lack of a co-ordinated marketing strategy and, perhaps most importantly, confusion regarding correct dosaging. For Cytotec®, the package insert includes only information about gastric ulcer doses and does not include any information about the safe dosages for reproductive health indications. This has led to frequent dosaging errors, with excessive dosages of misoprostol being commonly used for the second and third trimester indications. The danger of this is uterine rupture, and there is a concern that the wide availability of misoprostol in South Africa might have caused an increase in the numbers of uterine ruptures. The availability of evidence-based information or guidelines about misoprostol uses for various reproductive health indications is therefore an essential requirement for safe and effective use of misoprostol. An expert group convened by WHO met in Bellagio, Italy in 2007 to advise on optimal misoprostol dosages (the 'Bellagio Expert Group'). The resulting guidelines were published in a supplement to the *International Journal of Gynaecology and Obstetrics* in 2007 and are the basis for the dosage recommendations included in this review (Figure 1). The guidelines are also available on an independent website, http://www.misoprostol.org.

First trimester termination of pregnancy (induced abortion)

In the last two decades, medical TOP has become a safe alternative to vacuum aspiration and dilatation and curettage (D&C). Recently, the mifepristone/misoprostol regimen has become more widely available and is now considered to be the gold standard for early pregnancy termination.[4] Furthermore, a recent WHO study has demonstrated that reducing the time interval between the mifepristone and misoprostol doses from 48 to 24 hours has no effect on efficacy. The success rates to achieve complete abortion were 92.4 and 94.0 at 48 and 24 hours, respectively.[5] Increasingly, the evidence is pointing towards the use of sublingual or buccal misoprostol rather than vaginal: not only are these routes more acceptable to women, but they are associated with lower infection rates.

The regimen is also very successful when used without the mifepristone, achieving complete termination rates of 80–90%. A recent study compared a single dose of 800 mcg vaginal misoprostol to suction evacuation under local anaesthetic and found the success rate to be similar (94 and 95%, respectively), but with fewer side effects in those taking misoprostol.[6] The route of administration of misoprostol is important. Vaginal appears to be the most effective, followed by sublingual with oral being the least effective.[7,8] Sublingual misoprostol needs a more frequent administration, every 3 hours, to achieve a similar effectiveness to the vaginal route, but may be more acceptable to women. Therefore, for TOP, misoprostol should be administered vaginally unless there are reasons to avoid it. The recommended regimen is 800 mcg of vaginal misoprostol administered every 12 hours for a maximum of three doses.[8]

Early fetal demise (missed abortion)

The traditional treatment for early fetal demise has been suction curettage. However, several studies have shown that medical treatment is a safe, effective and acceptable alternative.[9] Both oral and vaginal 800 mcg misoprostol are highly effective and acceptable for treatment of early fetal loss, but the mean time for expulsion is shorter in the vaginal group.[10] Sublingual misoprostol is also effective, but has increased side effects at the same dosage.[11] However, a lower dosage of 600 mcg achieves similar efficacy at 86%.[12] It is unclear whether the addition of mifepristone gives any added benefit.[11] Therefore, the currently recommended regimen is 800 mcg vaginal misoprostol every 3 hours (max two doses) or 600 mcg of sublingual misoprostol every 3 hours (max two doses).[13] The woman should be reassessed after 14 days as it may take this long to be effective.

Incomplete miscarriage (abortion)

A single dose of 600 mcg oral misoprostol has been shown to be as effective as manual vacuum aspiration (MVA) in the management of incomplete miscarriage with complete evacuation rates of 95–99% after 1–2 weeks of follow-up.[14–17] It is also more acceptable to women than MVA. The use of double doses of oral 600 mcg misoprostol

Table 1. Summary of recommendations from the World Health Organisation on the use of misoprostol for various reproductive health indications

	On WHO model list of essential medicines[62] for this indication?	Text of WHO advice
First trimester		
Termination of pregnancy (induced abortion)	YES	'The following methods are preferred for early abortion: – Manual or electric vacuum aspiration – Medical method of abortion – a combination of mifepristone followed by a prostaglandin such as misoprostol or gemeprost, for up to nine completed weeks since last menstrual period. Misoprostol is the prostaglandin of choice for most settings since it is cheap and does not require refrigeration.'[20]
Early fetal demise (missed abortion)	NO	Formal recommendations not available for this indication.
Incomplete miscarriage (abortion)	YES	No formal recommendations available for this indication, but WHO textbook teaches use of MVA, with misoprostol or oxytocin as adjuncts.[63]
Second trimester		
Termination of pregnancy (induced abortion)	YES	'...the following methods are preferred: – Dilatation and evacuation (D&E), using vacuum aspiration and forceps; – Mifepristone followed by repeated doses of a prostaglandin such as misoprostol or gemeprost; – Prostaglandins alone (misoprostol or gemeprost), in repeated doses.'[20]
Third trimester		
Intrauterine fetal death	NO	No formal recommendations available for this indication, but WHO textbook teaches use of expectant management, prostaglandins, misoprostol, oxytocin or transcervical Foley.[63]
Induction of labour	YES	Formal recommendations not available for this indication, but WHO textbook teaches use of oxytocin, prostaglandins and transcervical Foley, with misoprostol only in specific situations.[63]
Prevention of postpartum haemorrhage	NO	'Skilled attendants should offer oxytocin for prevention of PPH in preference to oral misoprostol. Misoprostol can be used where no skilled birth attendants are available (without the other active management components)'[45]
Treatment of postpartum haemorrhage	NO	Formal recommendations not available for this indication, but WHO textbook only recommends oxytocin ergometrine and prostaglandin F2α – misoprostol is not mentioned.[63] Formal WHO guidelines available soon.
Gynaecology		
Cervical priming	NO	Prior to uterine evacuation misoprostol, mifepristone or gemeprost are 'recommended for durations of pregnancy over nine completed weeks for nulliparous women, for women younger than 18 years old and for all women with durations of pregnancy over 12 completed weeks'[20]

increases the rate of side effects without clinical benefits.[18] Misoprostol has recently been added to the WHO model essential drugs list for this indication. The recommended regimen is a single dose of 600 mcg oral misoprostol for women with a uterine size equivalent to 12 weeks or less.[19] The single dose should be given between 1 and 2 weeks to work.

Second trimester termination of pregnancy (TOP, induced abortion)

Surgical termination of early second-trimester pregnancy can be performed by D&C or suction curettage, whereas termination performed late in the second trimester requires cervical dilatation and fetal extraction. This can result in severe

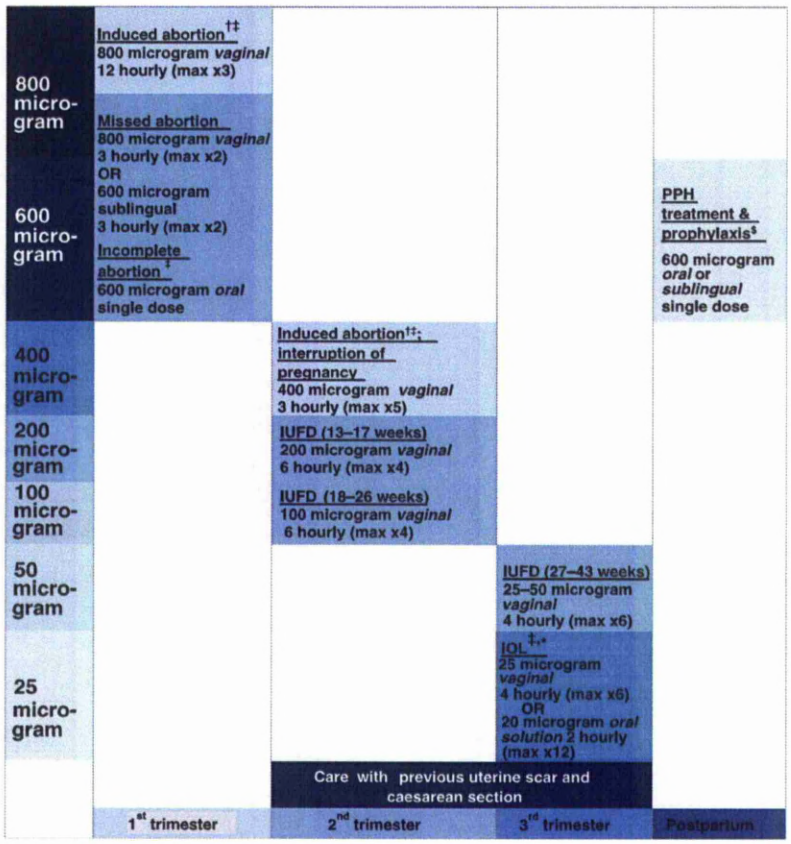

Figure 1. Recommended doses of misoprostol (IUFD: intrauterine fetal death; IOL: induction of labour; PPH: postpartum haemorrhage. †Where legal; ‡ included in the WHO Model List of Essential Medicines; * make sure you use the correct dosage – overdose can lead to complications!; $ oxytocin is first line as it is more effective than misoprostol).

complications including cervical laceration, uterine perforation and injuries to abdominal organs. Medical TOP is potentially safer and provides a good histological specimen which is important if the termination is for fetal malformations. Therefore, medical termination using mifepristone followed by a prostaglandin analogue is recommended by WHO[20] and the Royal College of Obstetricians and Gynaecologists.[21]

Misoprostol appears to be also effective when used alone and a variety of regimens with 200–600 mcg of vaginal misoprostol at different time intervals have shown similar effectiveness. A randomised trial of three regimens of misoprostol for second trimester induction at 14–30 weeks showed that 400 mcg of vaginal misoprostol 6 hourly provided the optimal regimen, with a shorter delivery interval than the 200 mcg dose and fewer side effects than the 600 mcg dose.[22] In balancing efficacy and side effects, a regimen of 400 mcg vaginal misoprostol at 3 hour intervals using a maximum of five doses is therefore recommended.[23] Higher doses may be needed for early second trimester TOP and lower doses may be sufficient to induce abortion later in the second trimester.

Uterine rupture is a rare but serious complication of TOP in the second trimester, occurring largely in grand multiparous women and those with a previous caesarean section. Further research is needed before misoprostol can be recommended as the standard method for second trimester TOP in women with a previous caesarean section, and the Bellagio group recommend caution in these women.

Intrauterine fetal death

There are a wide variety of clinically effective misoprostol regimens for the induction of labour following second and third trimester intrauterine fetal deaths (IUFDs). The required amount of misoprostol not only decreases with increasing gestational age, but has also been found to be lower in women where the fetus has died in utero.[24] Between the vaginal, sublingual and oral routes, sublingual misoprostol has the shortest induction to expulsion interval.[25] Oral misoprostol (400 mcg) acts more rapidly than

vaginal misoprostol (200 mcg), but has more side effects.[26] However, all completed within 48 hours. The vaginal route is therefore recommended for the treatment of IUFD. As a result of increasing uterine sensitivity to prostaglandin through pregnancy, the recommended doses are variable according to gestation.[27] From 13 to 17 weeks, 200 mcg vaginal misoprostol is required 6 hourly (four doses maximum), 100 mcg of vaginal misoprostol 6 hourly (four doses maximum) is recommended for 18–26 weeks and 25–50 mcg every 4 hours (maximum six doses) is used for a gestational age of over 27 weeks. If the first dose does not produce effective contractions, the second dose can be doubled.[27] Because of the risk of uterine rupture, it is recommended that women with a scarred uterus should receive lower doses of misoprostol, and not double the dose if there is no initial response.

Induction of labour with a live fetus

Induction of labour can be achieved by many medical and mechanical methods. Vaginal dinoprostone is the current gold standard method for cervical ripening and induction of labour, although misoprostol has been used as an alternative since 1987. The Cochrane review (70 trials containing over 10 000 women)[28] found that vaginal misoprostol is more effective than dinoprostone and oxytocin or oxytocin alone for induction, but rates of uterine hyperstimulation and meconium stained liquor were increased at doses over 25 mcg. Whether the latter is secondary to uterine hyperstimulation or due to a direct effect of misoprostol on the fetal bowel is not yet known. Recent pharma-sponsored studies have shown that both 25 mcg vaginal tablets and a sustained release pessary have similar efficacy to dinoprostone.[29,30] Vaginal misoprostol 25 mcg given 6 hourly is therefore recommended.

Oral misoprostol is also highly effective and has the benefit of a convenient route of administration. Overall, the meta-analysis of oral misoprostol trials (56 studies of over 11 000 women) shows it to be more effective than dinoprostone, but with higher rates of hyperstimulation.[31] This appears to be due to the doses in many of the trials being too high – the same effect is not seen with lower doses. Doses of 50 mcg orally or less have similar efficacy and hyperstimulation rates to dinoprostone.[32]

Obtaining an accurate dose of 20 or 25 mcg misoprostol by cutting the 100 mcg or the 200 mcg tablets can be difficult and imprecise. Even though misoprostol is evenly distributed throughout the tablet, cutting the tablet only achieves a dosage within 10% of the planned dosage in 58% of the time.[33] Although suboptimal, this should be compared with the alternative in low-resource settings which is an oxytocin infusion run without an electronic pump where dosages obtained are within 10% of planned in only 15% of observations.[34] To improve precision, an oral misoprostol solution can be made by dissolving one tablet (200 mcg) in 200 ml of tap water. A solution of 20 mcg of oral misoprostol (i.e. 20 mls of solution) given every 2 hours for a maximum of 12 doses titrated against individual uterine response is associated with a lower incidence of uterine hyperstimulation, shorter induction to labour interval and lower caesarean section rate than vaginal dinoprostone.[31] Head to head comparisons of low dose oral and vaginal misoprostol are few, but one study has shown that 20 mcg oral misoprostol solution administered 2 hourly is as effective as vaginal misoprostol in achieving vaginal delivery, but with a much lower rate of uterine hyperstimulation.[32]

The recommended doses for induction of labour using misoprostol are therefore 25 mcg vaginal or 50 mcg oral every 4 hours for a maximum of six doses. Another option is 20 mcg of oral misoprostol solution 2 hourly for a maximum of 12 doses.[35] There is currently limited evidence regarding the safety of misoprostol for the induction in women with previous uterine scar. The risk of uterine rupture with the use of misoprostol in women attempting vaginal birth after caesarean section is reported between 6 and 12% of women.[36,37] Therefore, misoprostol is contraindicated in women who have a uterine scar.

Prevention of postpartum haemorrhage

Postpartum haemorrhage is the leading cause of maternal mortality in the developing world,[38] accounting for about 25% of all maternal deaths worldwide. Initial small randomised trials comparing oral misoprostol to standard oxytocic regimens suggested that misoprostol was as effective as oxytocic drugs for PPH prevention. However, a large multicentre trial of nearly 20 000 women comparing 600 mcg of oral misoprostol with 10 IU of oxytocin showed that the rate of severe PPH (>1000 mls) was higher in the misoprostol group (4 vs 3.0%).[39] Oxytocin is therefore now recommended over misoprostol as first line for PPH prophylaxis as a part of the active management of labour.[40]

The evidence as to whether misoprostol is better than placebo is however complex. Although the Cochrane review of all RCTs show no overall difference in efficacy,[41] misoprostol appears to be beneficial in community settings. Three studies have explored this. A double-blind randomised trial conducted in The Gambia using 52 traditional birth attendants compared 600 mcg of oral misoprostol to 2 mg oral ergometrine (used as a placebo). Although there was no difference in PPH rate, there were fewer women with a fall in Hb >2 g/dl in the misoprostol group.[42] A second community double-blind RCT was undertaken in a primary

health centre in Guinea-Bissau which demonstrated a marked reduction in the rate of severe PPH (≥1000 ml) with 600 mcg sublingual misoprostol.[43] And most recently, an RCT of 1620 women in India has shown that 600 mcg oral misoprostol reduced the rate of severe PPH by 50% compared with placebo.[44] The findings of these three trials show the effectiveness of misoprostol in low resource, community settings, where the PPH rate is very high and where there are no alternatives for prophylaxis or treatment. The reason for this is unclear but may relate to the differing length of the normal third stage in community and hospital settings. In hospital settings where active management of the third stage is used, there is a tradition of enthusiastic early controlled cord traction (CCT) timed to coincide with the peak level of oxytocin. If early CCT is used with misoprostol, the placental detachment (and haemorrhage) will occur before misoprostol is at effective serum levels. In community settings, however, the timing of CCT is usually later and is likely to coincide with the peak serum levels of misoprostol at 20 minutes. This may explain why misoprostol appears to be more effective in community settings.

Based on the above evidence, WHO currently recommends the use of oxytocin rather than misoprostol for the prevention of PPH as part of the active management of the third stage of labour.[45] However, they recommend the use of either oxytocin or misoprostol in settings where active management is not being practiced or where no skilled birth attendants are available. The dosage recommended by the Bellagio expert group is a single dose of 600 mcg of oral or sublingual misoprostol.[46] The dose should not be repeated for 2 hours.

Treatment of PPH

For a decade now, studies have generated encouraging evidence on the use of misoprostol as an emergency treatment for PPH. Three placebo-controlled RCTs, testing the additive effect of misoprostol when used in conjunction with standard oxytocics to treat PPH, have found favourable trends in blood loss reduction in the misoprostol arms.[47–49] However, a statistically significant reduction of blood loss was demonstrated only after conducting a meta-analysis of the combined data from these RCTs testing varying regimens of misoprostol (600 to 1000 mcg) as an adjunct to PPH treatment.[49,50] Only one published hospital-based RCT has offered evidence on the use of misoprostol as a first-line treatment.[50] In this open label trial, 64 women were randomised to receive either 800 mcg misoprostol rectally or standard oxytocics as standalone treatment for PPH. The results showed dramatic improvements with the use of misoprostol, although the study design has been criticised as being subject to bias. To date, there is not enough evidence to recommend replacing injectable oxytocics with misoprostol for the first-line treatment of primary PPH.[51] This appears to be supported by the results of two large RCTs which at the time of writing are available in abstract format only.

Given the wide variety of reported doses and the demonstrable safety of misoprostol 600 mcg orally from the large WHO prophylaxis RCT,[39] the Bellagio expert group recommended 600 mcg of oral or sublingual misoprostol for PPH treatment, but not as first-line treatment.[52] Again they recommend that a second dose should not be repeated before 2 hours.

Cervical priming before transcervical procedures

Cervical priming refers to the softening or dilation of the cervix before transcervical procedures. These include TOP, hysteroscopy, D&C, insertion of intrauterine devices, and endometrial biopsies. Cervical priming using gemeprost, laminaria or mifepristone can facilitate mechanical dilatation, shorten the operation time, reduce blood loss and decrease the frequency of complications.[53]

Misoprostol has also been used effectively for cervical priming in pregnant women with both oral and vaginal misoprostol shown to be useful prior to vacuum aspiration.[54] The dose of 400 mcg vaginal misoprostol administrated 3 hours before the procedures is recommended as an optimal dose.[55] However, the same regimens using misoprostol either orally or sublingually are equally effective[54,56] and have the advantage of being more convenient and more acceptable to women.[56] A single dose of 400 mcg vaginal or sublingual misoprostol 3 hours prior to transcervical procedures has been recommended by WHO and the Bellagio expert group.[20,53]

Side effects

Chills and/or fever

Chills and fever are fairly common in association with the use of misoprostol but are transient. Typical rates for oral misoprostol 600 mcg are 28 and 7.5% for shivering and temperature over 38°C, respectively.[57] Temperatures of over 40°C are associated with high doses of misoprostol (e.g. 800 mcg), shorter intervals and oral or sublingual administration. The fever is transient and responds to antipyretics and physical cooling.

Gastrointestinal side effects

Nausea, vomiting and diarrhoea are common adverse reactions of misoprostol intake, affecting about 35% of women.[23] Diarrhoea is the most common side effect and it is usually mild and self-limiting within a day; vomiting

usually resolves in <6 hours. The gastrointestinal side effects are more common after oral or sublingual administration.[58]

Abdominal cramps

Abdominal cramps usually develop within the first few hours and may start as early as 10 minutes after administration. NSAIDs can be given for pain relief without affecting the effectiveness of misoprostol.[59] Higher doses of analgesia are required in younger women, those in late pregnancy and with multiple doses of misoprostol.[60]

Uterine hyperstimulation and rupture

Hyperstimulation may result from the use of excessive or repeated doses of misoprostol when used for labour induction. However, with the low misoprostol doses now recommended (25 mcg vaginally or 20 mcg orally), the incidence of hyperstimulation is similar to that of dinoprostone at 4–12%.[28,31]

Uterine rupture is another concern with the use of misoprostol, and is discussed in the section of Induction of labour with a live fetus.

Fetal abnormalities

A variety of congenital abnormalities have been reported after an unsuccessful use of misoprostol for TOP, but the most commonly reported are Mobius Syndrome (congenital facial paralysis with or without limb defects), absence of the fingers, club foot and cranial nerve anomalies (affecting nerves V, VI, VII, and XII). However, the risk of fetal malformation after misoprostol use is low with an estimated risk of <1% among exposed fetus.[61]

Conclusion

In settings with limited access to health care, misoprostol is an important intervention that could reduce maternal deaths both directly and through the more cost-effective use of health services. Misoprostol is, however, a powerful drug that needs to be used with care. Evidence-based information about the safest regimens should be widely disseminated so as to prevent its inappropriate use.

Conflict of interest

The authors have no conflicts of interest to declare. AW runs the http://www.misoprostol.org website to promote the appropriate use of misoprostol, but this does not receive any support from the pharmaceutical industry.

Contribution to authorship

AE carried out the literature search and writing of the manuscript first draft. AW revised the manuscript. ■

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The effect of misoprostol on postpartum contractions: a randomised comparison of three sublingual doses

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Objective To compare the postpartum uterine activity and side effects of various doses of sublingual misoprostol and intramuscular oxytocin.

Design Single centre, randomised trial.

Setting Zliten Teaching Hospital in Libya.

Population Forty-nine women who did not receive oxytocics in labour and who delivered vaginally.

Methods Thirty-five women were randomised to receive 200, 400 or 600 mcg of sublingual misoprostol PPH prophylaxis immediately following delivery. These were compared with 14 consecutive women given 10 IU of intramuscular oxytocin. Immediately after placental delivery, a Koala intra uterine pressure catheter was inserted transcervically into the uterine cavity.

Main outcomes measures The uterine pressure (in Montevideo units) measured over 120 minutes. Other outcomes included temperature and measured blood loss.

Results Women's age, parity, gestational age and neonatal birth weight were not significantly different between the four groups. There was no difference in intrauterine pressure between the three misoprostol doses. However, the uterine pressure was significantly lower than oxytocin with all three doses for the first 10 minutes ($P < 0.008$) and significantly higher than oxytocin from 50 to 120 minutes ($P < 0.008$). A dose-related rise in the body temperature and chills was observed in the misoprostol groups, with 8.3%, 8.3% and 45% of women experiencing a fever >39 °C with the 200, 400, and 600 mcg doses respectively.

Conclusion Intramuscular oxytocin has the highest immediate post partum uterine activity. Lower doses of misoprostol may be as effective as high doses and with fewer side effects. Clinical outcomes with low-dose misoprostol should be further explored (ISRCTN97277056). The study number is for the RCT reported in this paper.

Keywords Fever, misoprostol, postpartum haemorrhage, sublingual, uterine contraction.

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Introduction

Postpartum haemorrhage (PPH) is the leading cause of maternal mortality in the developing world and is responsible for about 25% of all maternal deaths worldwide.[1] The most common cause of PPH is the failure of the uterus to contract after childbirth (atonic PPH) and active management of the third stage of labour is recommended to prevent this.[2,3] This involves, as a minimum, the administration of a uterotonic drug at delivery and controlled cord traction. Oxytocin is recommended as the first-line oxytocic for the prevention of PPH.[3,4] However, oxytocin requires refrigeration because it is unstable when exposed to high ambient temperatures. Furthermore, this drug must be given parenterally, which requires a skilled birth attendant and a continuous supply of sterile syringes and needles. Both of these are frequently unavailable in low-resource settings. About 99% of maternal deaths occur in low-resource settings where there are poor transportation systems and a lack of skilled birth attendants and emergency obstetrics services.[5] Hence, a major objective for reducing maternal deaths in poor areas is to find low-cost, effective ways to prevent and control PPH.

Misoprostol is an orally active prostaglandin analogue with uterotonic effects, and is an option for PPH prevention in low-resource settings because of its thermostability,

cost-effectiveness and ease of administration. There have been at least 36 trials that have studied misoprostol for PPH prevention using doses between 200 and 1000 μg and a variety of routes including oral, vaginal, sublingual and rectal.[6] It is, however, as yet unclear which gives the best balance of efficacy and safety. Given the relative ineffectiveness of misoprostol for PPH, the tendency has been to use high doses. There are, however, potential dangers in this. Shivering and pyrexia are commonly reported adverse effects, and hyperpyrexia of over 40°C has been reported, reaching an incidence of 36% in some population groups.[7] A recent systematic review recommends further research to find the optimal route and minimum effective dose of misoprostol for routine use for the prevention of PPH.[8] The authors suggest that the sublingual route is likely to be the most suitable because of rapid uptake, prolonged duration of action and greater bioavailability.

This study was devised to compare the effects of three sublingual misoprostol doses using measurement of postpartum intrauterine pressure as a surrogate endpoint to evaluate the uterine activity of these uterotonics. We also compared the adverse effects associated with each treatment. Data from a small cohort of women given intramuscular oxytocin prophylaxis are also shown for comparison with the misoprostol data. We hypothesised that low doses of misoprostol produce a similar strength of myometrial contraction to high doses.

Methods

The study population was made up of women who gave birth at Zliten Teaching Hospital (in Zliten, Libya) between July and December 2009. This is a government-funded, secondary referral hospital serving a population of 200 000 people with 4500 deliveries per year. Before the study, a small pilot study was conducted in a nearby teaching hospital (in Misurata) to test and perfect the study equipment and data acquisition methodology. The data from this pilot study were also used to calculate the sample size for the main study. At the end of this small pilot study, the researcher moved to Zliten Teaching Hospital and the ethical approval was amended accordingly. During the trial set-up period for the main study at this new site (pilot data analysis, recalculation of samples sizes and development of the randomisation schedule and envelopes), 14 women were recruited to an observational study at Zliten where women were treated with oxytocin alone. Once all the study instruments were in place, the main randomised study commenced and women entering the delivery suite when the researcher was present (usual working hours during the week) were invited to participate.

Women who were 18 years old or over, who had a spontaneous onset of labour and no risk factors for PPH were approached upon arrival at hospital in early labour and invited to participate in the randomised study. If they agreed, informed consent was obtained. Risk factors for PPH included a history of PPH in a previous pregnancy, a history of antepartum haemorrhage in the current pregnancy, a previous caesarean delivery, multiple pregnancy and polyhydraminos, Women with anaemia (haemoglobin < 10.0 g/dl) or maternal infection were also excluded (Figure 1). Intrapartum exclusions were augmentation with oxytocin, instrumental delivery or caesarean section.

Women who gave birth to babies weighing >4 kg were not randomised. To enable this, the baby was weighed immediately after delivery, and the study drug was given slightly later than originally described in the protocol so as to allow the neonate's weight to be obtained. If the birthweight was ≤4 kg then the randomisation envelope was opened and the study drug was administered. In all women the drug was administered within a minute of birth. This change to the original protocol, made at the request of the local committee, resulted in an inconsistency that required both delivery of the study drug at the point of delivery and knowledge of the birthweight before randomisation. To correct this inconsistency, it was decided to delay the drug administration for a maximum of 60 seconds to allow rapid weighing and randomisation as described. This was done in the knowledge that the main effect of misoprostol does not occur until 20–30 minutes after administration so the effect of a 1-minute delay was likely to be minimal. Furthermore, as the delay occurred in all randomised women as well as in the cohort given oxytocin, it would have no effect on between-group comparisons.

A commercial randomisation programme was used to produce a random list of allocations to three doses of misoprostol (www.sealedenvelope.com). The allocations were written on cards and placed in consecutively numbered sealed opaque envelopes by staff not involved in the study. Blinding was not possible because the treatment was provided by the researcher who was available at the time of the delivery to carry out the final selection of women and collection of data.

The delivery of the baby was left entirely to the midwives. Routine practice is to give the uterotonic at the delivery of the anterior shoulder and deliver the placenta using controlled cord traction. Once the baby is delivered, the cord is cut and the baby is weighed immediately. All babies are then transferred to the neonatal unit for 2 hours of observation, even if the birth and immediate neonatal period were completely normal. Once the woman is transferred to the postnatal ward (after 2 hours of observation on the labour ward), the baby rejoins her and she is then able to start breastfeeding. This routine was not changed for women in the study except that the study drug was given by the researcher immediately after the baby was

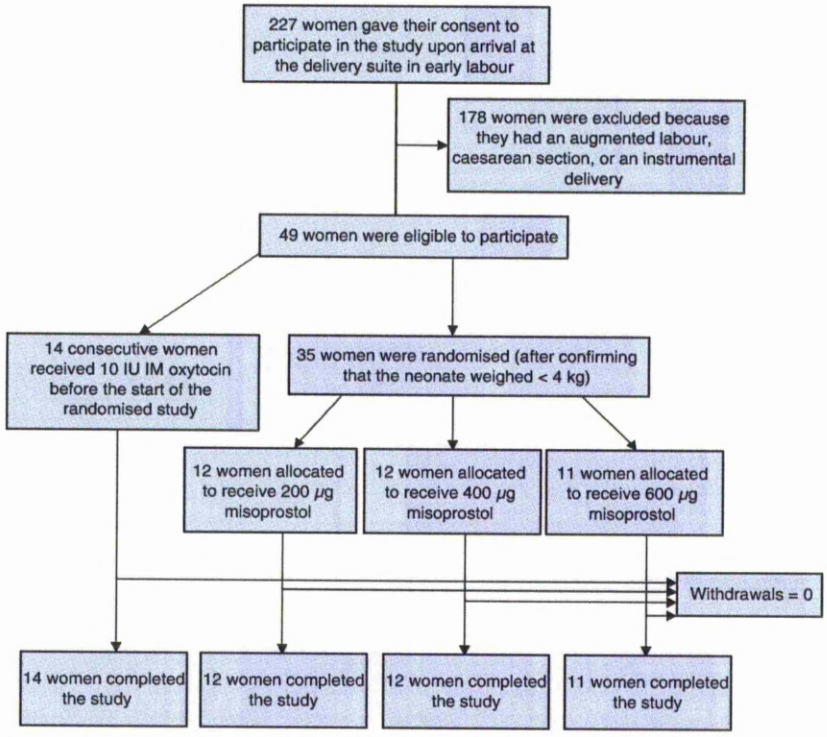

Figure 1. Flow diagram of the progress of the study.

weighed (which occurred within seconds of delivery) rather than at delivery. For women receiving misoprostol, the oxytocin was omitted.

Once the woman was determined as eligible, the next successive treatment envelope was opened and the designated treatment was given. We randomly allocated eligible women to 200, 400 or 600 μg sublingual misoprostol (Cytotec, Pfizer, Italy). The tablets were moistened with tap water before being placed under the tongue. At the same time, an 'under-buttocks drape with fluid-collecting pouch' (Kimberly-Clark, Kent, UK) was placed under the woman's buttocks for collection of any blood over the next 120 minutes. Immediately after placental delivery, an intrauterine catheter (Koala External Balloon Catheter IPC-5000E; Clinical Innovations, Salt Lake City, UT, USA; Figure 2) was inserted manually through the cervix into the uterine cavity until the tip of the catheter could be felt to touch the fundus. The catheter was secured in place with tape to the mother's thigh and connected to a Corometrics 118 maternal/fetal monitor (Corometrics Medical Systems Inc., Wallingford, CT, USA). The uterine activity was recorded and saved over the next 120 minutes. A researcher was with the women throughout the 2 hours of observation to record maternal temperature, pulse and blood pressure before labour, immediately after the delivery and then at 30, 60, 90 and 120 minutes. The women were closely observed for any adverse effects experienced. None of the women initiated breastfeeding until 2 hours after delivery, when they transferred to the postnatal ward.

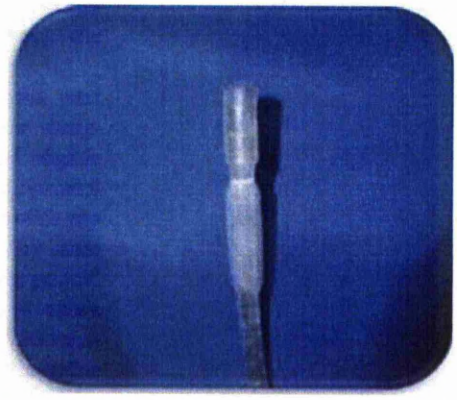

Figure 2. Koala External Balloon Catheter IPC-5000E (Clinical Innovations, Salt Lake City, UT, USA).

The primary outcome was intrauterine pressure over the first 10 minutes after delivery. The slight differences in time during which the pressure was measured in the first 10 postnatal minutes (because the catheter was only inserted after placental expulsion) meant that the Montevideo units (MVU) measurement had to be adjusted for the first

reading. This was done by adding together the uterine pressures collected in the first 10 minutes after delivery, dividing this by the length of time during which the measurements were taken (to get MVU per minute) and then multiplying by 10. The difference in means of the primary outcome was calculated using one way analysis of variance. The intrauterine pressures from 20 to 120 minutes postpartum were secondary outcomes.

Repeated measures of longitudinal data analysis (repeated measures analysis of variance) were used to compare the effect of the study treatments over 120 minutes of observation. Other mean comparisons used the one-way analysis of variance with multiple comparisons Bonferroni test. The incidence of shivering and fever and other adverse effects among the three misoprostol groups were compared using chi-square test with Yates' correction and Fisher exact test as appropriate. Statistical analysis was performed using Excel and PASW Statistics 17 (Excel, Microsoft Corp., Redmond, WA, USA; PASW 17, IBM Corp., Somers, New York, NY, USA). The difference between the four groups' parameters was taken as statistically significant when P values were <0.05. Based on the primary outcomes (mean intrauterine pressure over the first 10 minutes) and to detect a significant difference ($P = 0.05$, two-sided) at 0.8 power with 50% effect size, we needed a sample size of 12 in each group. The calculation was performed by G*Power 3.0.10 software (G*Power, Dusseldorf, Germany).

The study was approved by the University of Liverpool Ethics Committee (RETH000237) and accepted by the local hospital committees in Zliten and Misurata Teaching Hospitals.

Results

A total of 35 women were randomised out of a planned sample of 36. The study was curtailed before the planned finish when the investigator had to return to the UK. The randomised women were compared with a cohort of 14 women treated with oxytocin before the start of the randomised study. The age, parity, gestational age and baby's birthweight were similar in the four groups (Table 1).

All three doses of sublingual misoprostol produced a rapid increase in uterine activity with a peak at 40 minutes followed by a gradual decrease over the following 80 minutes (Figure 3). Throughout the observations, there was no significant difference between the intrauterine pressure in the three misoprostol dosage groups ($P = 0.8$).

The uterine pressure in those receiving oxytocin was highest in the immediate postpartum period and declined gradually after 40 minutes of administration. In the first 10 minutes, intrauterine pressures of the three misoprostol groups were significantly lower than those of the oxytocin group ($P = 0.008$). Conversely, the uterine pressure over the period from 50 to 120 minutes was significantly higher in the three misoprostol groups than in the oxytocin group ($P = 0.008$).

All the women were closely observed for adverse effects. Women who received intramuscular oxytocin reported no adverse effects (Table 2). In the misoprostol groups, the two most commonly reported adverse effects were shivering and hyperthermia, and a dose-related rise in the body temperature was observed. The incidence of severe hyperthermia (>39°C) was higher in the 600-μg misoprostol group than the 200- and 400-μg misoprostol groups, but this was not statistically significant ($P = 0.1$; Figure 4). Most of the women were not aware of the hyperthermia, but complained of coldness and marked shivering. All women in the 400- and 600-μg groups had shivering compared with 75% of the women in the 200-μg group. The differences were statistically significant ($P = 0.04$).

The blood loss was <500 ml in all women and was normally distributed. The lowest mean blood loss (±SD) was

Table 1. Basic characteristics of four treatment groups

	Oxytocin 10 IU IM	Sublingual misoprostol		
		200 μg	400 μg	600 μg
No. of women recruited	14	12	12	11
Parity, *n* (%)				
0	2 (14)	0	1 (8.3)	1 (9)
1	4 (28.5)	3 (25)	3 (25)	2 (18)
2	4 (28.5)	5 (41.5)	4 (33.3)	5 (45)
>2	4 (28.5)	4 (28.5)	4 (28.5)	3 (27.3)
Age (years)	28.5 ± 6.2	28 .1 ± 5.4	27 ± 3.3	26.3 ± 3.7
Gestational age (weeks)	40.2 ± 1.4	40.1 ± 1.1	40.3 ± 0.8	41 ± 1.1
Birthweight (g)	3350 ± 0.364	3458 ± 0.262	3595 ± 0.397	3522 ± 0.337

Date are mean ± SD unless otherwise stated.

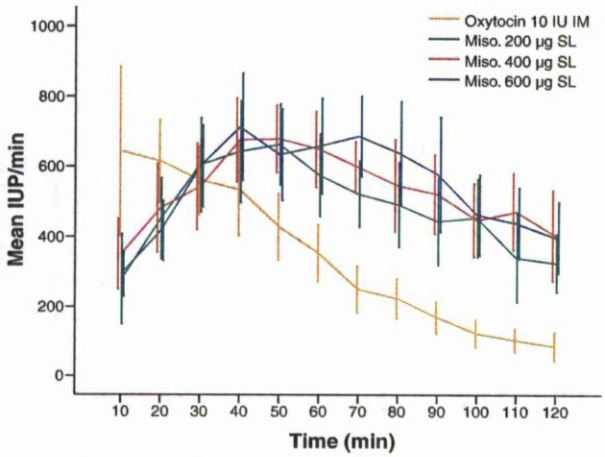

Figure 3. Line graph of mean postpartum uterine pressure in the different treatment groups. Data are presented as Montevideo units (MVU; ±SEM). The data in the first 10 minutes are adjusted to correct for the timing of placental delivery (MVU per minute).

Table 2. Incidence of adverse effects in the different treatment groups, *n* (%)

	Oxytocin 10 IU IM	Sublingual misoprostol		
		200 μg	400 μg	600 μg
No. of women recruited	14	12	12	11
Temperature >39°C	0	1 (8.3%)	1 (8.3%)	5 (45%)
Chills	0	9 (75%)	12 (100%)	11 (100%)
Coldness	0	9 (75%)	12 (100%)	11 (100%)
Abdominal pain requiring analgesia	0	0	2 (16.6%)	0
Nausea and vomiting	0	0	2(16.6%)	1 (9%)
Skin rash	0	0	0	1 (9%)

observed in women who received 10 IU oxytocin (193 ± 35 ml), followed by those who received 200 μg sublingual misoprostol (239 ± 46 ml), 600 μg sublingual misoprostol (275 ± 62 ml) and 400 μg sublingual misoprostol (299 ± 60 ml). The difference was statistically significant between oxytocin and 400 μg misoprostol ($P < 0.001$), oxytocin and 600 μg misoprostol ($P < 0.001$) and also between the three misoprostol groups ($P = 0.04$). None of the women received additional uterotonics or blood transfusion.

Discussion

This randomised trial was conducted to find out whether high-dose misoprostol offered any significant improvements in postpartum uterine pressure over low-dose misoprostol, and to compare the adverse drug reactions associated with each treatment. We also had concurrently collected data on intrauterine pressures following oxytocin administration, and this allowed us to compare the efficacy of these two types of oxytocics.

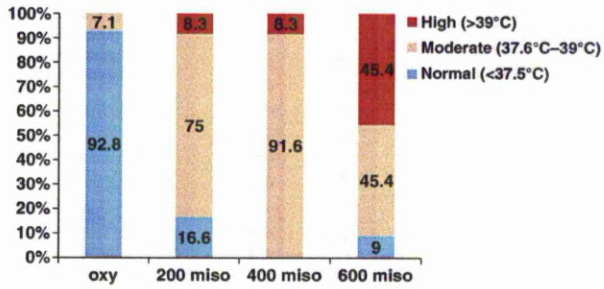

Figure 4. The incidence (%) of fever with oxytocin and 200, 400 and 600 μg sublingual misoprostol.

We used the Koala External Balloon sensor system[9,10] to measure uterine activity (Figure 2). All previous postpartum intrauterine pressure measurements have been conducted using a Galtec tipped catheter where a small pressure sensor is mounted into the tip of the device.[11–14] The Galtec tipped catheter was designed to measure intrauterine pressure in the first stage of labour. The pressure sensor is located in a recess within the head of the catheter, and it detects the myometrial contraction through an increase in the pressure of the surrounding amniotic fluid within the almost closed cavity. In the third stage of labour, however, the uterus is open and the amount of fluid is limited because it is squeezed out during each contraction. The Galtec tipped catheter therefore functions poorly in this situation. For accurate measurements it is logical for any measurement device to have a large surface area in direct contact with the adjacent uterine wall. The Koala catheter has a 3-cm air-filled balloon near its tip that connects directly to an external reusable transducer mounted into the connecting cable. The balloon sits in direct contact with the uterine wall and is compressed directly during the contraction.

The mean intrauterine pressure over 120 minutes for the intramuscular oxytocin and the three doses of sublingual misoprostol are shown in Figure 3. The difference seen in speed of onset of action can be explained both by the different pharmacokinetics of these drugs and by the different routes of administration. The absorption of misoprostol is affected by mouth dryness and any surrounding fluid;[15,16] we standardised for this by moistening the tablets with tap water before placing them under the tongue. The pharmacokinetics may explain our observation of stronger and more frequent initial uterine contractions with intramuscular oxytocin than with sublingual misoprostol. The peak of

uterine contraction for oxytocin was within the first 10 minutes after administration whereas for sublingual misoprostol it was only achieved after 30–40 minutes. This mirrors their plasma concentrations.[15,17]

Most uterine bleeding occurs immediately after placental separation.[18] At this time, the mean intrauterine pressure for intramuscular oxytocin was significantly higher than with the three sublingual misoprostol doses. Even though the effective action of sublingual misoprostol started late, it caused high uterine contractions maintained over a considerable period of time. This may help to prevent steady moderate bleeding, which may be unobserved until serious hypodynamic manifestations occur.

Very few studies have examined the effect of misoprostol on uterine activity during the third stage of labour using measurement of intrauterine pressure as an indicator. Chong *et al.*, using a Galtec tipped catheter, found no difference in postpartum uterine pressures between women given oral misoprostol and intramuscular syntometrine.[11,12] The readings with this catheter, however, may not be reliable for the reasons outlined above.

The mean intrauterine pressure of the three different doses of sublingual misoprostol was not significantly different over the 2-hour observation period. This is consistent with previous research findings. For example, in one previous study there was no difference in intrauterine pressure measurements with five different doses of oral misoprostol, although again this used a Galtec tipped catheter.[11] Furthermore, randomised control trials found that both 600 μg sublingual and 400 μg oral misoprostol were more effective than placebo for the prevention of PPH,[19,20] and a meta-analysis in which an indirect comparison was made between the 400 and 600 μg oral doses concluded that the two were likely to be equally effective.[21] As a result of adverse effects associated with high doses of sublingual misoprostol, some researchers have argued that the dosage of misoprostol should be reduced from the 600 and 800 μg doses in common usage today. Our research findings give further weight to that argument.

The most commonly observed adverse effects in this study were shivering and hyperthermia. Around half of the women who received 600 μg had a temperature >39°C whereas the incidence for women who received 400 and 200 μg was around 8%. Most of the women were not aware of the hyperthermia, but complained of coldness and intolerable shivering (all women in the 400 and 600 μg groups had shivering). Although there appeared to be clinical differences in the incidence of hyperthermia between high and low doses, the difference was not statistically significant because the study was not powered enough to detect differences in incidence of adverse effects. Shivering and hyperthermia have been reported in most of the studies using misoprostol for different indications and with variable routes and doses. In a recent multicentre randomised control trial using 800 μg sublingual misoprostol for treatment of PPH, the overall incidence of fever (>40°C) was 14%. However, the incidence of fever varied between different populations with the highest incidence in Ecuador (36%) and the lowest (0%) in Egypt.[7,22] In our study, the incidence of adverse effects appeared to be dose-related. Prostaglandins are the principal mediator of fever in the brain and can pass the blood–brain barrier to the thermoregulation centres in the hypothalamus, causing elevation of the thermoregulatory set point. To increase the body temperature to the new set point, there are increases in heart rate, muscle tone and shivering.[23–25]

Clinical trials have shown that oxytocin prophylaxis is more effective than oral misoprostol for the prevention of blood loss >1000 ml.[26] Given that most blood is lost around the time of placental expulsion during the first 10 postpartum minutes, it is not surprising that oxytocin is the more effective prophylactic.[17] It is only after the first 50 minutes that the uterine contraction strength was higher in the misoprostol groups. However, although the effect of misoprostol is delayed, it should be eventually as effective as oxytocin in preventing massive blood loss through atony. There is evidence for this from a Cochrane Review in which there are significant differences between oxytocin and misoprostol in blood loss >500 and >1000 ml, but no difference in need for blood transfusion.[6]

We acknowledge that this study recruited only low-risk women in whom the chance of developing an atonic PPH was small. However, even in a study that included high-risk women, the majority of women would not develop an atonic PPH—and this is the very group for which the intervention is designed. However, the area of interest in this study is the comparison between drug dosages and, as such, it is important to have as homogeneous a group of women as possible. This was best achieved through the use of a low-risk group. Furthermore, it was considered by the ethical committee to be inappropriate to expose women at risk of PPH to misoprostol, which has been shown to be less effective for prophylaxis than oxytocin.

Conclusion

In conclusion, our results showed that 200, 400 and 600-μg doses of sublingual misoprostol produced similar levels of uterine activity, but that the severity of adverse effects was dose-related. These findings suggest that lower doses of misoprostol may be as effective as high doses. Clinical applications of low doses of sublingual misoprostol for the prevention of PPH should be further explored by large randomised trials comparing the effectiveness and the safety of low doses of sublingual misoprostol.

Disclosure of interests

The authors have no conflicts of interest to declare. AW runs the independent www.misoprostol.org website to promote the appropriate use of misoprostol, but this does not receive any support from the pharmaceutical industry.

Contribution to authorship

AE participated in the study design, carried out the study procedure and wrote the manuscript. MSE assisted with running the study in Libya and the final approval for publication. MOE and OAE assisted with the recruitment of the participants and data acquisition of the study in Libya. AW designed the study, revised the manuscript providing comments on its intellectual content and helped draft the manuscript and the final approval for publication.

Details of ethics approval

The study was granted ethical approval from the University of Liverpool ethics committee (RETH000237) and was accepted by the local hospitals in Libya on 14 July 2009 and 24 August 2009.

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Acknowledgements

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Journal club

Discussion points

1. Background: Compare the evidence with regard to therapeutic versus prophylactic use of misoprostol for postpartum haemorrhage (PPH). Include in your evaluation data (or arguments) on effectiveness, safety and cost-effectiveness. Would your answer be different depending on the population and setting?
2. Methods: Explain why the authors might have been compelled to exclude women at high risk for PPH.

   The researchers used a blood-collecting pouch for estimation of blood loss; contrast this to other methods, including visual estimation.

   What is the Bonferroni method? When is it desirable?
3. Results and implications: Describe the different effects of misoprostol and oxytocin on uterine pressure, with reference to Figure 3.

   Discuss the consequences of misoprostol-induced hyperthermia (Figure 4).

   Discuss the external validity (generalisability) of the results; are they likely to stimulate further research, change in clinical practice, or both?
4. Publication issues: The authors have reported no conflicts of interest. Discuss the need for such disclosures, particularly for trials of medicinal products, with reference to the *Uniform Requirements for Manuscripts Submitted to Biomedical Journals*, as published by the International Committee of Medical Journal Editors (www.icmje.org/ethical_4conflicts.html. Describe other instances in the research process where such disclosure may be required.

Read the *BJOG* editorial policy with regard to ethical approval for studies in countries without local ethics committees or institutional review boards (www.bjog.org/view/0/editorialPolicy.html). How did the authors deal with this issue? Discuss the advantages and disadvantages of their approach. ■

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Suggested further reading

Blum J, Winikoff B, Raghavan S, Dabash R, Ramadan MC, Dilbaz B, et al. Treatment of post-partum haemorrhage with sublingual misoprostol versus oxytocin in women receiving prophylactic oxytocin: a double-blind, randomised, non-inferiority trial. *Lancet* 2010;375:217–23.

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EDITORIALS

Misoprostol for the management of postpartum haemorrhage

No benefit if oxytocin is available, but useful where no other alternatives exist

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For decades, oxytocin and ergometrine have been the treatments of choice for postpartum haemorrhage caused by ineffective uterine contraction (uterine atony). Although both drugs are effective, oxytocin is more widely used because it has fewer side effects and can be used safely in women with hypertension and pre-eclampsia. However, in their usual form both drugs can be given only by injection, and both require refrigeration. They are therefore of limited availability and benefit in low resource settings, especially in rural areas. Misoprostol, an orally active and heat stable prostaglandin E_1 analogue, has therefore emerged as a popular alternative. Until a year ago, there was limited evidence for its ability to treat postpartum haemorrhage.[1 2] However proponents have argued that it should be "parachuted in" to high risk areas despite the lack of evidence.[3] This, in part, has been responsible for its inclusion in multiple guidelines on postpartum haemorrhage both in rich and poor settings (despite the call in a systematic review for more studies[2]).

Since the systematic review of the treatment of postpartum haemorrhage was last updated in 2007,[2] three large double blind randomised trials have been published.[4-6] Few research teams had been able to carry out a randomised trial of treatment for this condition, but Gynuity Health Projects and the World Health Organization, with backing from the Bill and Melinda Gates Foundation, were able to recruit more than 80 000 women in 14 centres worldwide to three trials to define the role of misoprostol. Although these trials are impressive, they have gone largely unnoticed by many maternity care workers.

The first compared 800 µg sublingual misoprostol with 40 IU oxytocin given in a litre of intravenous solution over 15 minutes for the treatment of postpartum haemorrhage in women who had not received oxytocin prophylaxis.[4] The study recruited 9348 subjects; 10% of them were diagnosed with postpartum haemorrhage (around 700 ml of blood loss) and received the study treatments. Further bleeding of at least 300 ml (1 L total) occurred in 30% of the women given misoprostol and in only 17% of women given oxytocin (relative risk 1.78, 95% confidence interval 1.40 to 2.26). Misoprostol was associated with more side effects—"intolerable shivering" was seen in 11% of women receiving misoprostol compared with less than 1% of women taking oxytocin (55.2, 7.70 to 397).

The second trial used the same protocol but in women who had received routine prophylaxis with oxytocin.[5] Evidence of the benefit of prophylaxis with oxytocin was overwhelming—only 3% (809/31 055) of women bled compared with 10% in the trial above where no prophylaxis was available. In this second trial, additional blood loss of 300 ml or more after treatment was similar in the two groups (34% *v* 31%; 1.12, 0.92 to 1.37), whereas blood loss of more than 1 L after treatment occurred in 11 (3%) women managed with misoprostol and three (1%) women given oxytocin (3.62, 1.02 to 12.89). Intolerable shivering occurred in 4% and less than 1% of women treated with misoprostol and oxytocin respectively (16.8, 2.25 to 125). These findings suggest that 800 µg sublingual misoprostol is a possible alternative to 40 IU intravenous oxytocin for the management of postpartum haemorrhage after prophylactic oxytocin, but that it does have more side effects.

The third study assessed the effect of using misoprostol in addition to conventional injectable uterotonics to treat postpartum haemorrhage.[6] The study compared 600 µg sublingual misoprostol to placebo in 1422 women who were being treated with 10 IU intramuscular or slow intravenous oxytocin for the treatment of postpartum haemorrhage. The study found no significant difference between the two treatment groups in the proportion with blood loss of 500 ml or more within 60 minutes (14% in both treatment groups; 1.02, 0.79 to 1.32) or blood loss greater than 1000 ml (1% in both treatment groups; 1.02, 0.41 to 2.55). Consistent with the other trials, side effects were more common with misoprostol than with placebo.

Following on from WHO studies nearly 10 years ago showing that misoprostol was less effective than oxytocin for prophylaxis,[7] the results of these studies were disappointing for misoprostol enthusiasts. Not only is it less effective than oxytocin, but it has more side effects and no adjunctive effect if the woman has already been given oxytocin. The only comfort is that detailed examination of the data, along with the excellent outcomes for the participants, suggests that misoprostol is better than nothing.

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So is there any remaining role for misoprostol in the management of postpartum haemorrhage? In settings where oxytocin is freely available it should be used instead of misoprostol for prophylaxis. And although the two drugs have similar efficacy after oxytocin prophylaxis, there is no benefit of providing a second drug that is commonly more expensive, has more side effects, and has no additional effect.

In rural low resource settings, however, where injectable oxytocics are rarely available, misoprostol is an important weapon in the fight against postpartum haemorrhage related mortality. Its heat stability and ease of use mean that all midwives and doctors in these settings should carry a stock. Furthermore, recent observational studies in women having home births in Nepal and Afghanistan suggest that giving misoprostol to women antenatally for self administration immediately after delivery may be a safe and effective strategy.[8 9] A large placebo controlled randomised trial is now under way to test this hypothesis. If true, this would provide an effective self administered treatment for the first time to those women most at risk of death from postpartum haemorrhage, and it could help reduce maternal mortality worldwide.

Competing interests: All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work; AW runs an independent non-profit making website (www.misoprostol.org) that seeks to disseminate guidelines on the optimal doses for misoprostol use (it does not receive any funding or sponsorship); he also has an ongoing collaboration with Gynuity Health Projects and has received funding from them to set up a randomised trial of misoprostol for management of postpartum haemorrhage in women living in rural Uganda; AE declares no conflicts of interest.

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Short communication

Historical trends in the timing of informed consent for research into intrapartum complications

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Obtaining informed consent for clinical trials involving the management of intrapartum complications is complex. This article describes the strategies used to obtain consent over the last 60 years using data from the Cochrane Library. Of 138 intrapartum randomised studies, 37% had no record of the consent procedures. Of the remainder, 74% sought consent only when the complication developed, 11% sought consent from all women in early labour, and 13% gave all women antenatal information and then sought written consent when the complication arose. Despite the existence of ethics guidelines for intrapartum consent, many studies fail to follow their advice.

Keywords Clinical trials, Cochrane Library, intrapartum research, informed consent.

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Introduction

Informed consent has been described as being at the 'heart of ethical research'.[1] For consent to be valid, three critical factors need to be present: the individual needs to be well informed, be 'competent' enough to give their agreement to participate, and not be coerced. Meeting these criteria in labouring women who have developed an intrapartum complication is challenging. Not only is a woman likely to be anxious and distressed from labour pains, but there is often little time to provide detailed information about the trial. There is also a risk of coercion as women may fear being treated poorly or being abandoned should they refuse participation.[2] For these reasons, it has been suggested that all potentially eligible women should be approached antenatally and the consent procedures should be completed at that time, even though only a small percentage of them will end up eligible for inclusion. Consumer groups, however, have expressed concern that detailed discussions antenatally about rare complications could cause anxiety and medicalise completely normal pregnancies.[1] Striking the right balance between these two strategies is a major problem for those conducting intrapartum research.

There is very little information on women's preferences in this situation. An insight can be obtained from those having epidural analgesia as a clinical intervention, and whether they feel able to understand the risks before giving consent. In one study, the majority of the women wanted to hear about all the potential epidural risks and for these risks to be discussed with them before the onset of labour.[3] They also found that the labouring woman's pain and anxiety levels (including several other factors such as age, education level and opioid premedication) did not correlate with her ability to understand information. Although there have been no direct examinations of intrapartum consent strategies, women in the USA entering a 21-year cohort study were asked about the collection of data and samples during labour.[4] They expressed concern about the level of intrusiveness caused by researchers collecting data during labour and wished to minimise intrapartum contact with researchers.

Children were previously thought to be the 'therapeutic orphans' when it came to clinical research. However, with recent legislation and guidance many would now agree that pregnant women are the new 'therapeutic orphan' subgroup. Despite research bodies and regulators acknowledging the need for research in this group, there remains a paucity of

specific guidance. Perinatal researchers have looked towards guidance available for 'vulnerable populations', but this is not particularly suitable because pregnant women do not strictly fall under this definition. Instead, the regulations pertaining to emergency care may be more appropriate. Good ethical research practices for pregnant women fall somewhere between the regulations and guidance for vulnerable populations and emergency research, but neither are currently suitable.

Until recently, there has been little ethical advice available for researchers conducting intrapartum research. In 1997, two consumer groups [the Association for Improvement in the Midwifery Services (AIMS) and the National Childbirth Trust (NCT)] published their *Charter for Ethical Research in Maternity Care.*[1] They recommend the distribution of information to all women antenatally with signed consent only once the complication develops. Recently, the Royal College of Obstetricians and Gynaecologists (RCOG)[5] has published advice on how to obtain valid intrapartum consent for research. They agree with the AIMS/NCT system, although they recommend varying the method according to the nature of the study and likelihood of the event occurring. With a high risk of occurrence (10–100%, e.g. perineal tears), they suggest that full informed consent should be gained from all potentially eligible women antenatally and confirmed at the time of the complication for those who develop it.[5] With rarer complications (1–10%, e.g. retained placenta) they recommend that women should receive outline trial information antenatally, but then obtain full information and provide signed consent only once the woman becomes eligible during labour. For very rare complications (<1%, e.g. shoulder dystocia) it is appropriate to provide information only once the event occurs or when the suspected risk exceeds the background risk of 1 in 100.

Many women in labour have a heightened sense of emotion and some may feel very vulnerable. To make a clumsy approach to them at this time to participate in a research project may result in anxiety, confusion and distraction, and may threaten the crucial bond of trust that develops between a labouring woman and her carers. The guidelines from the RCOG are therefore welcome as a way of avoiding this situation, but it is not known how frequently they are currently used.

This study examines the approaches to obtaining informed consent that researchers have used over the past five decades, and describes the current frequency of different consent strategies.

Methods

The Cochrane Database of Clinical Trials was searched and 15 reviews (see Table S1) were identified that examined therapies for the management of intrapartum complications (i.e. those that arose for the first time in labour and could not be predicted previously). The Cochrane search method is described elsewhere[6] but includes weekly searches of MEDLINE and hand searches of 30 journals as well as the proceedings of major conferences.

All randomised controlled trials within these reviews (i.e. those involving the management of intrapartum complications) were included for analysis. Duplicates, studies available in abstract form only, those not in English, trials involving the study of prophylactic interventions, unpublished trials and those studying non-obstetric emergencies were excluded. Studies that fulfilled the criteria were examined and information from these studies was entered into an electronic proforma. The consent methods were classified into nine separate categories: *consent not needed* (no consent required by the ethics committee), *unclear method* (method of consent unclear from the published manuscript), *no data* (no evidence within the published manuscript of any consent having been taken), *post-event* (no consent taken at time of recruitment, but consent was obtained post-event), *proxy* (no personal consent, consent from proxy only), *consent at complication* (consent at time of complication with no previous information provided), *antenatal information with consent at complication* (antenatal information in groups, leaflets or individually with formal consent sought at the time of complication), *early labour* (consent from all women during early labour), and *AN* (consent from all pregnant women during the antenatal period). Information on the consent approach used in the trial was examined and classified independently by two independent investigators (DP and NS) and checked by a third (AE, GV or ADW). The final classification was confirmed through majority agreement.

The results were analysed according to decade of publication and stage of labour at which the complication occurred.

Results

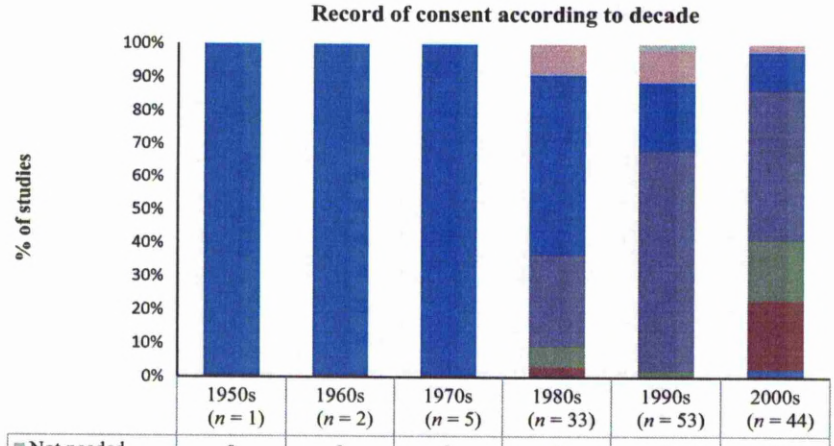
Three hundred references were retrieved from the 15 Cochrane reviews, but only 138 met the inclusion criteria (see Figure S1 for flow chart). Of these, 37% had no clear record of the consent procedures. Of the remainder, 74% sought consent for the first time at recruitment when the complication developed, 11% sought consent from all women in early labour so that consent for the trial was obtained in advance for those who developed the complication and 13% gave all women antenatal information and then sought consent only when the complication arose. In one study the researchers obtained consent from all women in the antenatal period, and in another the ethics committee did not deem it necessary to obtain written consent.

The standard of reporting changed through the decades (Figure 1). None of the eight studies conducted before 1980 recorded any evidence of consent having been taken, but by the 2000s 86% of studies had clear descriptions of consent procedures. Between 1980 and 2010 a variety of consent methods were used, with antenatal information-giving (and consent at the time of complication) and consent from all women in early labour becoming increasingly common. In the 1980s and 1990s, 23% and 59% of studies, respectively, obtained consent from women at the time of the complication. In the 2000s the level fell with only 45% of researchers obtaining consent in this way. A variety of other methods were used instead: 20% of researchers obtained consent from the total population during admission to the labour ward and 18% provided antenatal information and then obtained consent at the time of the complication.

In those studies in which consent procedures are described, taking consent from women when complications arise is by far the most common method for consent in the first and second stages of labour (79% and 82%, respectively, see Figure S2). For third-stage complications, however, only 38% of studies sought consent using this process; 46% sought consent from all women in early labour and 15% gave all women information antenatally and then sought formal consent at the time of the complication.

Discussion

Although there are a variety of methods described for obtaining informed consent in the 138 studies discussed in this review, most researchers simply sought consent at the time of complication. However, the method of obtaining consent appears to have been improving since the 1980s, with the introduction of novel methods of obtaining informed consent. Deficiencies remain. In the decade since 2000, only 18% of studies used the method advised by the consumer groups, and 11% of studies did not provide any information at all about their method of obtaining informed consent in the published manuscript. These statistics are disappointing and suggest that researchers need to be made more aware of the current intrapartum consent guidelines provided by consumer groups and, more



	1950s ($n = 1$)	1960s ($n = 2$)	1970s ($n = 5$)	1980s ($n = 33$)	1990s ($n = 53$)	2000s ($n = 44$)
Not needed	0	0	0	0	1	0
Unclear	0	0	0	3	5	1
No data	1	2	5	18	11	5
Post-event	0	0	0	0	0	0
Proxy	0	0	0	0	0	0
Comp	0	0	0	9	35	20
AN info & comp	0	0	0	2	1	8
Early lab	0	0	0	1	0	9
AN	0	0	0	0	0	1

Figure 1. Consent approach according to decade of the trial. Definitions: *Not needed*, no consent required by ethics committee; *Unclear*, method of consent unclear; *No data*, no evidence of consent recorded; *Post-event*, no consent at time of recruitment, obtained post-event; *Proxy*, no personal consent, consent from proxy only; *Comp*, consent at time of complication with no other information provided; *AN info & comp*, antenatal information in groups, leaflets or individually with formal consent sought at the time of complication; *Early lab*, consent from all labourers during early labour; *AN*, consent from all pregnant women during the antenatal period.

recently, by the RCOG. Although following these guidelines would require some additional resources, the use of these consent guidelines would provide researchers with an ethically and legally respected methodology. The simplest method of enforcing their use would be through the ethics research committees.

To our knowledge this is the first published review of historical intrapartum research consent methodology. Its strength is that it examined every published English language randomised trial on the management of intrapartum complications in great detail. This was ensured by the use of the robust study identification methodology of the Cochrane Library database and the use of triple review for each manuscript. However, this study did not examine all the randomised controlled trials on the management of intrapartum complications. Studies published in abstract form only or not in English were excluded. This may have led to an over-optimistic view of the global nature of consent methodology over the last 60 years. Furthermore, this review has not examined research on trials of intrapartum analgesia, because pain was not considered to be a complication of labour. This has meant that a number of studies on epidural analgesia have not been included for analysis.

The ability of any individual to take in new information and make informed decision choices while in labour can vary hugely. Dorantes et al.[7] found that 41% of women refused participation in an anaesthesia trial simply because they felt the discomfort of labour pains hindered their ability to understand the trial information. Conversely, 59% believed that the discomfort did not hinder their ability to take part. The nature of the complication also determines the speed with which consent needs to be obtained. For example, obtaining consent for a study of the treatment of postpartum haemorrhage would have much less time than for a study of a therapy for delay in the first stage of labour in women with epidural analgesia. It could be argued therefore that a single consent process for all women is inappropriate. Vernon et al.[8] attempt to address this by the use of the midwife or doctor as a 'gatekeeper' to assess the emotional and physical state of the labouring woman and therefore determine whether she is 'competent' to give informed consent to the researchers. They also argue that large amounts of antenatal information should be provided in a variety of formats, and then each woman can access it to a degree appropriate to her needs.[8] This advice has since been incorporated into the RCOG guidelines.[5] However, despite arguments questioning the competency of labouring women, there is evidence that some of the events of labour may not interfere with a woman's capacity. Jackson et al.[3] found that none of the anticipated variables (including pain, anxiety and opiate use) correlated with the labouring woman's ability to understand epidural risks. They concluded that women in labour are just as capable to give informed consent as at other times. However, when evaluating epidural risks, the woman is also anticipating a major benefit (pain relief), which may act as a positive pressure for the woman to provide consent.

In this study we also examined how consent approaches varied with the stage of labour. In most first-stage and second-stage trials, consent was usually sought at the time of the obstetric complication development. van Lier and Roberts[2] recommended that consent could be sought in early labour (<4 cm dilatation) because most women are relatively comfortable during this stage and the intervals between contractions do allow for adequate explanation and discussion. In contrast, the second stage of labour may be characterised by considerable pain and exhaustion. All the randomised trials for second stage complications in this review were comparing operative delivery methods for failed progress in labour. This time is especially difficult because the situation is rapidly evolving, the mother is exhausted and there is often concern about fetal wellbeing. Despite this, most studies still used consent at the time of complication. The degree to which consent could be seen to be valid in this situation would be questioned by many of today's ethics committees.

In the third stage of labour a variety of research methods have been used, to some extent reflecting the conditions the studies are concerned with. In studies of postpartum haemorrhage treatments there was no time to take consent, and studies obtained consent from all potentially eligible women in early labour. In contrast, in the event of a perineal tear or retained placenta there is a little more time to discuss consent and so researchers sought consent at the time of the complication.

A major factor affecting the consent method is the year in which the research was conducted, with this review finding major changes over time. There have been numerous drivers for this including the increased use of ethics committees. In the UK, for example, although research ethics committees were established in the NHS in the mid-1960s, reporting to them was initially voluntary. In the early 1990s it became a requirement for all proposed clinical studies to obtain approval from an ethics committee, a factor that will have increased the use of formal and ethical consent procedures. The requirement for formal approved processes increased further with the European Union Clinical Trials Directive and the Research Governance Framework for Health and Social Care. This increase in UK research governance has not been seen in all countries, and the stringency and demands of ethics committees now vary widely between countries.

The seeking of consent from all eligible women in early labour, and the provision of antenatal information with consent at the time of complication are the fastest emerging strategies for intrapartum consent. The latter strategy is

in line with the recommendations of AIMS,[1] the North-West Clinical Trials Network[8] and the recent RCOG guidelines (which more specifically tailor the degree of antenatal information provision to the incidence of the complication).[5] The benefits of this strategy have not however been formally assessed and qualitative research into women's preferences for intrapartum research consent methods are urgently needed. Research consent strategies have never been subject to debate outside the narrow confines of intrapartum research groups. Although limited attempts have been made to address this question in emergency medicine,[9] none have examined intrapartum consent. If we are to identify a strategy that is safe, respectful of women's autonomy and which allows intrapartum research to continue, then it will be necessary to initiate a public debate involving women, ethicists, researchers and consumer groups, and not just take it for granted that the professionals know best.

Disclosure of interests
None.

Contribution to authorship
ADW had the original idea for the study and assisted with data analysis and writing of the paper. He is the guarantor of the paper. DP and SN collected the data, conducted the primary analysis and wrote the first draft of the paper. AE and GV assisted with the analysis and reviewed the drafts of the manuscript. All authors approved the final version of the manuscript before publication.

Details of ethics approval
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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow chart showing how studies were excluded from analysis.

Figure S2. Chart showing the consent approaches in relation to the stage of labour.

Table S1. Cochrane database reviews of treatment for intrapartum complications that were examined in this review.

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